

M 2021

MASTER IN BIOENGINEERING

**P-cadherin as a phenotypic stability factor of the
hybrid epithelial/mesenchymal phenotype in
breast cancer**

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September 15, 2021

Agradecimentos

O começo de um novo capítulo na vida de uma pessoa pode ser tanto entusiasmante como assustador.

Assim que comecei esta jornada percebi que, enquanto indivíduos, somos os únicos criadores do nosso próprio caminho. Tendo por base esse pensamento, acredito ainda que é sempre melhor termos companhia ao longo do nosso percurso. Por isso, gostaria de agradecer a todas as pessoas que me apoiaram, talvez com a mesma loucura que eu, quando decidi entrar nesta aventura.

À Professora Joana Paredes, o meu muito obrigada. Agradeço-lhe a oportunidade de fazer parte deste grupo incrível, o “Cancer Metastasis”, e de poder trabalhar numa área que tanto gosto e tanto carinho tenho. Muito obrigada pelo apoio, oportunidades e orientação que me deu ao longo deste percurso e, acima de tudo, obrigada por ter acreditado em mim e nas minhas capacidades. Agradeço ainda que nunca me tenha deixado desmotivar, mesmo quando os resultados não eram os esperados, e por me mostrar que há sempre uma explicação.

Ao meu co-orientador, Doutor Tito Jesus, agradeço toda a ajuda, conhecimento e liberdade para puder crescer e tentar resolver os problemas autonomamente. Obrigada por me deixares pensar e desenvolver as minhas ideias, ouvires as minhas dúvidas e, obrigada por todo o brainstorming que, de uma forma ou de outra, sempre deu resultados práticos importantes.

À Doutora Ana Sofia Ribeiro, pela simpatia, conhecimento, disponibilidade e, acima de tudo, pela grande ajuda e paciência principalmente nas análises bioinformáticas.

À Doutora Bárbara Sousa um muito obrigada pela incansável ajuda. Sem dúvida foste a ajuda certa na altura certa e não podia estar mais grata pela orientação que me deste.

À Rita, pela forma amiga com que sempre me incentivou. Por ter sido um apoio tão grande desde o início e pelo carinho e disponibilidade para me ajudar, mesmo fora de horas! Agradeço-te também todas as brincadeiras, gargalhadas e bom ambiente que trazes contigo todos os dias para o laboratório. Obrigada

por me mostrares que só erra quem trabalha e por me fazeres ver que, na ciência, a prática e a teoria podem ser duas coisas bem distintas.

Aos meus colegas de laboratório e ao meu grupo, o “Cancer Metastasis”: Inês Conde, Irina Fonseca, Maria Miguel, Cíntia Horta, Rita Canário e Carolina Noronha agradeço-vos todos os bons momentos que partilhamos. Obrigada pelo companheirismo, espírito de equipa, brainstorming e, acima de tudo, por me receberem tão bem e se disponibilizarem sempre para me ajudar em qualquer altura.

À Bia, por me ouvir e apoiar, por estar sempre ao meu lado, por todo o apoio ao longo dos vários anos que nos conhecemos e pela amizade incondicional. Obrigada por me fazeres ver que por mais que a vida tenha os seus altos e baixos não vamos querer viver sem arriscar.

À Irina, à Mariana e à Marta, agradeço todo o companheirismo, amizade e todas as conversas sobre tudo e nada. Obrigada por também vocês estarem sempre presentes em qualquer altura e por todos estes anos de amizade.

À Catarina, à Kika e à Tânia agradeço a amizade incondicional e a presença e apoio nas horas mais difíceis. Não podia ter feito esta caminhada de cinco anos com melhor companhia que a vossa. Nem todos os momentos foram fáceis, de todo que não, mas com vocês aprendi que viver de maneira descomplicada é a melhor forma para ser feliz. Muito obrigada por serem muito mais do que amigas.

Ao Nuno, por todos estes anos de amizade, por também tu estares apenas à distância de uma chamada, e por me ouvires e apoiares sempre, incondicionalmente.

Às minhas queridas Tias, Teresa, Margarida e Maria João, pelo apoio e carinho desde sempre e em tudo. Pela amizade e companheirismo, pelas risadas e por todas as vezes que foram e são muito mais do que tias. E, nestes últimos anos, pelo nosso querido grupo “Paris” que tornou a distância um bocadinho mais perto.

Aos meus pais, pelas oportunidades que me deram desde sempre e por me incentivarem nos momentos mais difíceis. Pelo apoio e confiança, pois mesmo

quando eu não acreditava ser capaz vocês acreditavam em mim. Por me deixarem crescer enquanto pessoa e permitirem que seguisse o meu caminho, mesmo que nem sempre concordassem.

Ao meu irmão, pelo companheirismo, incentivo, apoio, motivação, cumplicidade e amizade. Por seres o meu maior defensor em tudo e, acima de tudo, por me mostrares o quão bom é ter um irmão.

Aos meus Avós, à minha família, e a todas as pessoas que passaram na minha vida e deixaram um bocadinho de si, pois sem vocês também não me teria tornado a pessoa que sou hoje.

Resumo

A Transição Epitélio-Mesenquimal (EMT) é um processo fisiológico pelo qual células de origem epitelial adquirem um fenótipo mesenquimal. Durante anos, assumiu-se o EMT como um processo binário, onde as células apenas transitavam entre estes dois fenótipos, o epitelial (E) e o mesenquimal (M). Contudo, novas evidências sugerem a existência de estados intermediários onde características epiteliais e mesenquimais se podem observar, levando ao estabelecimento de fenótipos híbridos. Além disso, apesar destes fenótipos híbridos não se encontrarem ainda bem caracterizados, verificou-se que, quando adquiridos por células tumorais, se associam a uma maior agressividade e a um pior prognóstico para os doentes.

A P-caderina (P-cad), uma molécula de adesão célula-célula, codificada pelo gene *CDH3*, tem sido associada a vários tumores, sendo que a sua sobre-expressão induz invasão celular de forma coletiva, assim como características estaminais e resistência a *anoikis*.

Apesar da expressão de P-cad ser considerada um fator de mau prognóstico em cancro de mama, pouco se sabe sobre o papel desta caderina no processo de EMT. O objetivo deste trabalho consiste assim na investigação da P-cad como fator de estabilidade do fenótipo híbrido em células de cancro de mama, uma vez que funcionalmente a sua expressão induz capacidade invasiva e características estaminais, apesar da manutenção da adesão celular.

Através de uma análise bioinformática, identificámos 13 genes EMT significativamente correlacionados com a expressão de *CDH3*. Nas linhas celulares de cancro de mama, podemos observar que a expressão de P-cad promoveu a expressão de marcadores epiteliais, bem como a expressão dos fatores de transcrição ZEB2 e Δ Np63. Relativamente aos marcadores mesenquimais, assim como aos marcadores de fenótipo híbrido pAMPK e GRHL2, a sobre-expressão da P-cad induziu uma menor expressão destes.

A P-cad regula a expressão de diferentes marcadores de EMT, sendo ainda capaz de induzir alterações funcionais e fenotípicas. Este trabalho demonstra assim, que a P-cad deverá ter um papel determinante no EMT e na manutenção do fenótipo híbrido, independentemente do subtipo molecular de cancro de mama.

Abstract

The biological process by which epithelial cells acquire a mesenchymal phenotype is called epithelial-to-mesenchymal transition (EMT). For many years, it was believed that EMT was a binary process, where cells would only transit between two stable phenotypes, epithelial (E) and mesenchymal (M). New evidences have demonstrated the existence of intermediate or hybrid EMT phenotypes (*hy-phen*), where epithelial and mesenchymal characteristics can be observed. Importantly, cancer cells harboring the *hy-phen* were reported to play a crucial role in metastasis, by promoting cellular plasticity, cancer stemness, collective migration and drug resistance.

P-cadherin (P-cad) is a cell-cell adhesion glycoprotein, encoded by the *CDH3* gene, that is related to several tumors, since its overexpression induces collective cell invasion, as well as stem cell features and *anoikis*-resistance.

Despite the role of P-cad expression in patient's poor prognosis and the *in vitro* evidence of P-cad expressing tumors being more therapy resistant, aggressive and with stem-like properties, little is known about the relevance of this glycoprotein during EMT. Thus, our goal is to study whether P-cadherin can act as a biomarker of an early stable hybrid E/M phenotype, operating as a lever of phenotypic cell plasticity that drives metastatic aggressiveness.

Through a bioinformatics analysis, we identified 13 EMT genes significantly correlated with *CDH3*. We observed that, in the different breast cancer cell lines, P-cad expression promoted the expression of epithelial markers, as well as the transcription factors ZEB2 and Δ Np63. For mesenchymal markers, and hybrid EMT markers pAMPK and GRHL2, P-cad overexpression induced a lower expression of these.

To conclude, P-cad can regulate the expression of different EMT markers, being also able to induce functional and phenotypic changes. Thus, this work demonstrates that P-cad can play an important role in EMT and in the maintenance of the *hy-phen*, regardless of the breast cancer subtype.

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Abbreviations List

AJ	Adherens junctions
ALDH	Aldehyde dehydrogenase
AMPK	Adenosine monophosphate-activated protein kinase
BCT	Breast-conserving therapy
BLBC	Basal-like breast cancer
BRCA	Breast cancer associated gene
BSA	Bovine serum albumin
CDH1	E-cadherin gene
CDH2	N-cadherin gene
CDH3	P-cadherin gene
cDNA	Complementary DNA
CTCs	Circulating tumor cells
DNA	Deoxyribonucleic acid
DCIS	Ductal carcinoma <i>in situ</i>
E-cad	Epithelial cadherin
E-markers	Epithelial markers
EpCAM	Epithelial cell adhesion molecule
ECM	Extracellular matrix
EMT	Epithelial-to-mesenchymal transition
FDA	Food and Drug Administration
FBS	Fetal bovine serum

GRHL2	Grainyhead Like Transcription Factor 2
HER2	Human epidermal growth factor receptor 2
hy-phen	Hybrid phenotype
IBC	Inflammatory breast cancer
ITG α V/CD51	Integrin α V
ITG β 3/CD61	Integrin β 3
ITG β 4/CD104	Integrin β 4
LRR	Locoregional recurrence
M-markers	Mesenchymal markers
MET	Mesenchymal-to-epithelial transition
mRNA	Messenger RNA
N-cad	Neural cadherin
NAC	Neoadjuvant chemotherapy
NRF2/NFE2L2	Nuclear factor (erythroid-derived-2)-like 2
NSM	Nipple-sparing mastectomy
Ovol2	Ovo Like Zinc Finger 2
P-cad	Placental cadherin
PARP	Poly (ADP-ribose) polymerase
PBS	Phosphate buffered saline
PSF	Phenotypic stability factor
PDL	Programmed death ligand
PMRT	Postmastectomy radiation
RNA	Ribonucleic acid
Slug	Snail family transcriptional repressor 2

Snail	Snail family transcriptional repressor 1
SRF	Src family kinase
SSM	Skin-sparing mastectomy
TF	Transcription factor
TJ	Tight junctions
TNBC	Triple-negative breast cancer
Twist	Twist family bHLH transcription factor
VCAM1/CD106	Vascular cell adhesion molecule 1
WBI	Whole breast irradiation
ZEB1	Zinc finger E-box binding homebox 1
ZEB2	Zinc finger E-box binding homebox 2
ZO	Zonula occludens

1. General Introduction

Cancer is one of the most prevalent diseases worldwide, having, in 2020, been the leading cause of death of around 9.9 million people. It is a disease that affects a person's life in several ways, whether physically, mentally, or even socially. It is already associated to several risk factors such as: age, gender, family history, nutrition, alcohol consumption, obesity, radiation exposure, lack of physical activity, tobacco, genetic changes and race (Kapil, Bhadoria, Sareen, Singh, & Dwivedi, 2014; Sun et al., 2017).

Cancer is a genetic disease, which means that, regardless the causes that trigger its appearance – multifactorial inheritance – these will lead to the occurrence of changes in the cellular genome, in a specific type of genes called proto-oncogenes (Low, Zembutsu, & Nakamura, 2018). These oncogenic alterations usually happen due to the occurrence of gene mutations (germinative, spontaneous and/or induced) or due to epigenetic alterations that lead to the increase of oncogenes' expression (Ataollahi, Sharifi, Paknahad, & Paknahad, 2015). This process is quite common and can happen in almost every cell of the body. However, there are mechanisms to correct these alterations, which are controlled by proteins coded by tumor suppressor genes. These proteins are able to repair DNA errors, by slowing cell division or by inducing cell apoptosis (Morris & Chan, 2015). However, if failures also occur in these repair mechanisms, due to tumor suppressor genes' inactivation/mutation, a transformed or cancer cell can actually appear.

1.1. Breast Cancer

Breast cancer was the most diagnosed cancer worldwide in women during 2020, with about 2.3 million of new cases diagnosed, and immediately followed by lung and colorectal cancer (Sung et al., 2021; WHO, 2021). In Portugal, according to data from the Portuguese League Against Cancer (<https://www.ligacontracancro.pt/>), approximately 6.000 new cases are detected annually and around 1.500 women die from this disease each year.

In cellular and molecular terms, breast cancer is a very heterogeneous disease. It includes several histological and molecular subtypes, and, for this reason, patients can be treated with different therapeutic approaches. Also, the

stage in which the disease is initially diagnosed plays an important role on the selected therapy, as well as on patient's prognosis (Perou et al., 2000). Tumors can be characterized according to their location within the mammary gland, being diagnosed as – carcinomas *in situ*, when restricted to the ducts or lobules, or as invasive carcinomas, if cancer cells already crossed the basement membrane and invaded adjacent tissues (Watkins, 2019). Moreover, due to transcriptomic studies (Creighton et al., 2010; Curtis et al., 2012; Provenzano, Ulaner, & Chin, 2018), it is known that breast carcinomas can be divided into different molecular subtypes, mainly due to their enrichment in estrogen signaling (Luminal tumors) or to the overexpression of the human epidermal growth factor receptor 2 (HER2) (HER2-overexpressing) (Figure 1), which have a clear impact on the prognosis and treatment of breast cancer patients.

The most frequent tumors (>70%) are classified as Luminal, A or B, depending on their proliferative capacity and resistance to endocrine treatment. Luminal A are less proliferative and more responsive to therapy compared to Luminal B, being the ones associated with a better prognosis. In addition, this subtype of tumors are the ones with the best prognosis (Gao & Swain, 2018; Lowery, Kell, Glynn, Kerin, & Sweeney, 2012). Another subtype is the HER2-overexpressing tumors, in which cancer cells overexpress the HER2 receptor on their surface. These tumors represent nearly 15-20% of breast carcinomas and are associated with a more aggressive phenotype and poor prognosis (Kreutzfeldt, Rozeboom, Dey, & De, 2020). There is also the Basal-like breast cancer subtype which includes tumors that are usually triple-negative (ER, PR and HER2 negative) and enriched in basal markers (Fragomeni, Sciallis, & Jeruss, 2018; Prat et al., 2015; Roulot et al., 2016). Importantly, this subtype is associated with a very poor prognosis and the mortality rate is about 40%, five years after diagnosis (Dent et al., 2007; Yin, Duan, Bian, & Yu, 2020).

In relation to therapeutic options, there are two types of local therapies with a high success rate when used in early stages of the disease: breast-conserving therapy (BCT) and mastectomy. The BCT approach involves only the removal of the tumor, followed by adjuvant whole breast irradiation (WBI), that is only performed when is possible to remove the entire tumor with considerable negative margins and to obtain an acceptable cosmetic result. Otherwise,

mastectomy is the best option and can be followed by breast reconstruction (J. Gu et al., 2018; Moo, Sanford, Dang, & Morrow, 2018; Recht & Solin, 2011). There are 3 types of mastectomy, depending on the part of the breast that will be removed. As the name suggests, total mastectomy leads to the removal of the breast parenchyma, nipple areolar complex and excess skin from the chest wall, thus, there is only enough tissue to close the incision. In the case of skin-sparing mastectomy (SSM), skin is left to facilitate the reconstruction process; whereas in the nipple-sparing mastectomy (NSM), the nipple areolar complex is also preserved in addition to the skin (Galimberti et al., 2017).

In patients with advanced disease, post-mastectomy radiation is essential due to locoregional recurrence (Lyons & Sherertz, 2014; Yun, Diaz, & Orman, 2018). Another therapeutic approach that is frequently used for inoperable tumors in advanced stages of the disease is neoadjuvant chemotherapy (NAC), which allows to restrict and limit the area of the tumor, facilitating its extraction during surgery (Pilewskie & Morrow, 2017). Often, after the surgical removal of the tumor, some adjuvant medical therapies can be administered systemically to the patient to eliminate micro-metastases (Thorn & Ladewig Hess, 2021).

In addition to local therapies, for many years two traditional therapies, chemotherapy, and radiotherapy, were frequently used to treat this disease. However, these do not only affect cancer cells but also healthy cells, leading to numerous adverse effects, from fatigue to a weaker immune system (Krug et al., 2017; Radecka & Litwiniuk, 2016). To overcome the adverse effects of traditional therapies, biologic, and targeted therapies, as well as endocrine therapies, have appeared.

Therefore, hormonal therapies, such as tamoxifen, fulvestrant, aromatase inhibitors and progestins, have been used in the treatment of Luminal tumors, since they are sensitive to these medicines (Ades et al., 2014; Harbeck & Gnant, 2017). In the case of HER2+ tumors, target therapies can be used due to their sensitivity to some monoclonal antibodies, like Trastuzumab (Lamb, Vanzulli, & Lanari, 2019; Oh & Bang, 2020). These new therapeutic approaches have significantly improved patient's outcome and prognosis (Escrivá-de-Romaní, Arumí, Bellet, & Saura, 2018). And in the last year, for TNBC, PARP inhibitors

have been approved to treat patients with mutations in the BRCA1/2 genes and, in 2019, the FDA approved the first checkpoint immunotherapy for TNBC patients expressing PDL1 (Cyprian, Akhtar, Gatalica, & Vranic, 2019; Slade, 2020).

Still, it is necessary to further investigate more about the disease and its biological functions in order to create new and more effective therapeutic approaches.

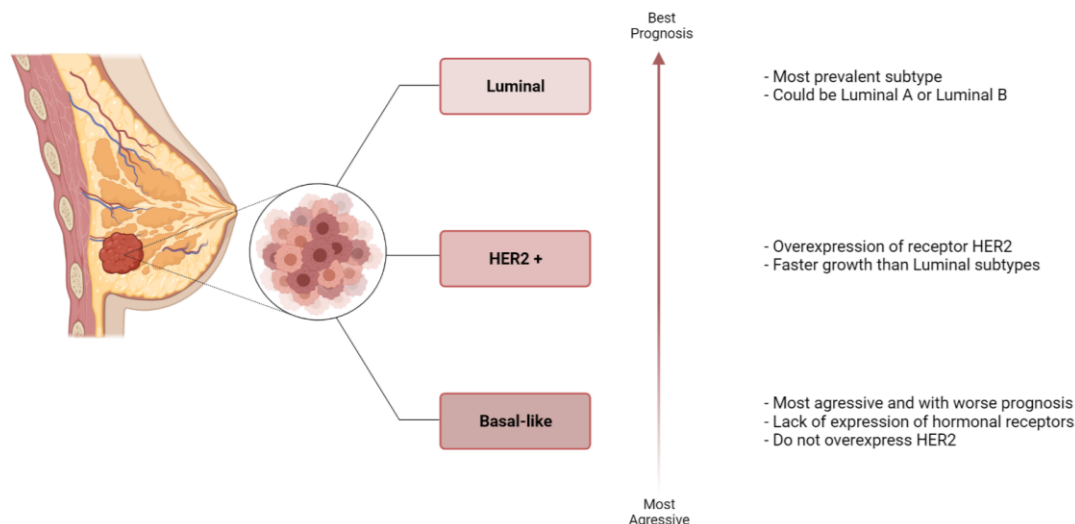


Figure 1. Breast Cancer Subtypes. Organization of the different subtypes of breast cancer depending on the expression of hormonal receptors and HER2 receptor; Prognosis association according to breast cancer subtypes. [Image created in Biorender].

1.2. Metastasis

It is well known that the existence of a lump or tumor within a breast is not the biggest problem associated with breast cancer. When diagnosed, a mammary tumor can be surgically removed, through a more or less invasive method, and it can be actually associated to a good prognosis for the patient. However, the major problem associated to breast cancer, as in most cancers, are the putative capacity of invasive cancer cells to colonize distinct organs and give rise to distant metastasis.

Metastasis consists on the dissemination of tumor cells from the initial tumor to adjacent tissues and distant organs (Seyfried & Huysentruyt, 2013), being a process that involves several steps (Figure 2). First, it is assumed that cancer cells from the initial tumor undergo several phenotypic alterations, allowing their detachment from the primary tumor through the ability to degrade the

extracellular matrix (ECM) and to migrate to adjacent tissues – **Invasion** (Figure 2). Secondly, due to chemotactic, physical and survival factors, cancer cells can migrate towards lymph and/or blood vessels, crossing their endothelial barrier and entering into circulation – **Intravasation** – where they start to be called as **Circulating Tumor Cells (CTCs)** (Figure 2). In the same way, CTCs can adhere again to the endothelium of vessels, pass through their wall and drawn out of them, in a process similar to diapedesis – **Extravasation** (Figure 2). Finally, cancer cells can be able to colonize tissues or organs, promote angiogenesis, and form a new tumor in a distinct microenvironment. This dynamic process is extremely aggressive to tumor cells and only a small percentage of them will be able to complete all these steps. However, if they are able to do it in a successful way, a metastasis of the primary tumor will be formed. Actually, metastases are often more aggressive than the primary tumor, since there is the selection of cancer clones with specific characteristics that allow their plasticity and survival in distinct organs other than breast (Bacac & Stamenkovic, 2008; Chambers, Groom, & MacDonald, 2002; Duffy, McGowan, & Gallagher, 2008; Lambert, Pattabiraman, & Weinberg, 2017; Welch & Hurst, 2019).

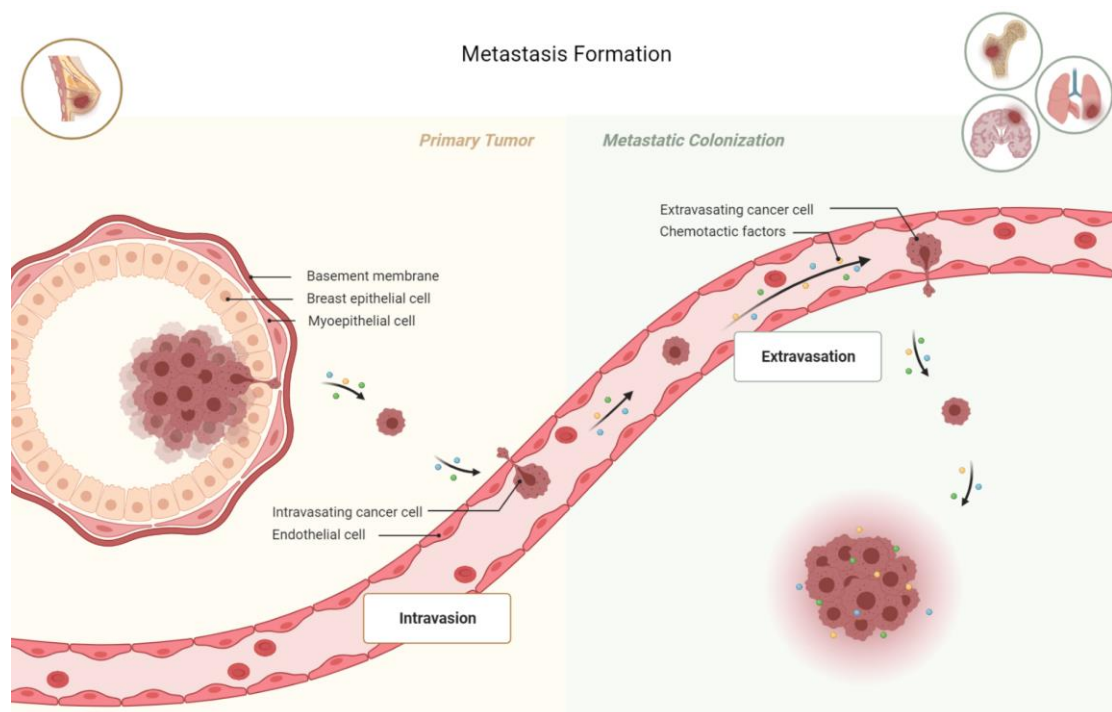


Figure 2. Overview of breast cancer metastasis. Cancer cell begins to detach from the primary tumor and, due to chemotactic factors, invades the vessels, where remains in circulation until it is attracted to a distant organ or tissue, colonizing it and forming a secondary tumor. [Image created in Biorender].

1.2.1. Circulating Tumor Cells (CTCs)

As previously reported, metastases are the leading cause of death in breast cancer patients (Agarwal, Balic, El-Ashry, & Cote, 2018; Petri & Klinge, 2020; Piñeiro, Martínez-Pena, & López-López, 2020). As also referred, metastases are caused by CTCs, which are tumor cells, with high metastatic potential (Fabisiewicz & Grzybowska, 2017), that invade the bloodstream and systemically disseminate to different parts of the body, until they invade other tissues or organs, where they will form a new tumor. Due to their high proliferative capacity (Kallergi et al., 2013; Müller et al., 2005), stem-like properties (Al-Hajj, Wicha, Benito-Hernandez, Morrison, & Clarke, 2003; Ponti et al., 2005; Theodoropoulos et al., 2010) and ability to form tumors, these cells can be considered cancer stem cells (García, Weitz, & Schölch, 2018). Furthermore, in circulation, together with CTCs, there are other circulating components, such as exosomes and soluble factors, which are essential to allow the development of metastases by preparing metastatic niches in distant organs (Eble & Niland, 2019; L. Zhang & Yu, 2019).

Interestingly, CTCs can be found isolated, which is more often, or in clusters. Clusters are circulating tumor cells that are linked through cell-cell junction proteins and which usually show collective migratory capacity (X. Liu et al., 2019; Padmanaban et al., 2019). Although their existence is rare, these CTC clusters have a higher metastatic potential compared to individual CTCs (Aceto et al., 2014), being their presence significantly associated to a worse prognosis for cancer patients.

1.3. Epithelial to Mesenchymal Transition (EMT)

The biological process by which cancer cells lose their epithelial characteristics to become invasive is called epithelial-to-mesenchymal transition (EMT), which is a reversible process, being the mesenchymal-to-epithelial transition (MET) its reverse (Bakir, Chiarella, Pitarresi, & Rustgi, 2020; T. Chen, You, Jiang, & Wang, 2017). This molecular process is not only involved in cancer progression, but also in tissue repair and embryonic development (Gonzalez & Medici, 2014; Stone et al., 2016; Thiery, Acloque, Huang, & Nieto, 2009). Due to

this phenomenon, cancer cells undergo several molecular, biochemical and morphological changes that allow them to acquire a new phenotype and functional behavior (Nieto, Huang, Jackson, & Thiery, 2016). Therefore, cancer cells pass from an epithelial to a mesenchymal phenotype, acquiring stem-like properties and cell plasticity, experiencing significant alterations in cell-cell and cell-matrix adhesion. In consequence, cancer cells lose their apical/basal polarity and gain migratory and invasive capacity, as well as resistance to apoptosis and therapies, such as radio and chemotherapy (Jolly et al., 2019; Micalizzi, Farabaugh, & Ford, 2010; Nieto, 2013).

For many years, it was believed that EMT was a binary process, where cells would only transit between two stable phenotypes. The **epithelial phenotype**, where cells show apical/basal polarity and great cell-cell and cell-matrix adhesion, are not motile and express epithelial (E) markers. And the **mesenchymal phenotype**, in which cells show an elongated shape, are migratory and invasive, and express mesenchymal (M) markers (Werner, Stenzl, Pantel, & Todenhöfer, 2017; S. Wu et al., 2015) (Figure 3). In that sense, it has been always assumed that cancer cells would need to go through EMT to become invasive and metastatic.

Interestingly, it has been found that isolated CTCs from liquid biopsies from cancer patients can be epithelial or mesenchymal (Soler et al., 2018; Woo & Yu, 2018) and that CTC clusters are also formed either by epithelial and mesenchymal cells. This means that CTC clusters express different markers and can respond to different external stimuli, which ends up giving them a great resistance, and enhancing invasion and metastasis (K. J. Cheung & Ewald, 2016; K. J. Cheung, Gabrielson, Werb, & Ewald, 2013).

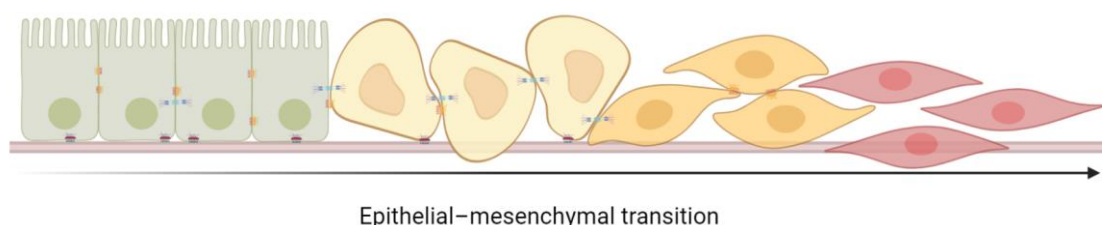


Figure 3. Epithelial-mesenchymal transition (EMT). Epithelial cells undergo phenotypic changes, where they lose cell-cell adhesion and epithelial (E) markers, and acquire mesenchymal characteristics, such as mesenchymal (M) markers, an elongated shape and migratory and invasive capacity. [Image created in Biorender].

1.3.1. Epithelial Markers

Epithelial cells, as mentioned above, are round-shaped and cohesive, and express epithelial markers, such as **E-cadherin (*CDH1*/E-cad)**, **β -catenin (*CTNNB1*)**, **occludin (*OCLN*)**, **zonula occludens-1 (*TJP1*/ZO-1)** and **EpCAM (*EPCAM*)**. These markers are mainly transmembrane and cytoplasmic glycoproteins, expressed at tight junctions (TJ) or adherens junctions (AJ) and which maintain cell-cell adhesion and the anchorage to the actin cytoskeleton.

E-cad is a transmembrane glycoprotein, calcium-dependent, expressed in all epithelial cells, which has a crucial role in the formation of AJ and in the maintenance of cell-cell adhesion (Coopman & Djiane, 2016), cell polarity and integrity of epithelial tissues (van Roy, 2014). A decrease in E-cad expression or loss is often associated to poor patients' prognosis in many cancers, including breast cancer (Horne et al., 2014; Jie et al., 2013; Xing et al., 2013). However, this molecule is more than an adhesive protein, since it also regulates cell cycle progression, differentiation, dissemination, and cell fate (Wong, Fang, Chuah, Leong, & Ngai, 2018). Actually, E-cad is considered a tumor suppressor protein and the loss of its expression, in association with EMT, frequently occurs in metastatic cells, mediated by EMT transcription factors that repress the *CDH1* gene and, consequently, E-cad expression (Na, Schecterson, Mendonsa, & Gumbiner, 2020; Saitoh, 2018; Valastyan & Weinberg, 2011). However, recent studies have shown the presence of E-cad in metastasis, promoting cell adhesion and cluster migration (Elisha, Kalchenko, Kuznetsov, & Geiger, 2018; Hapach et al., 2021; Padmanaban et al., 2019).

Catenins in general, such as **β -catenin**, are cytoplasmic molecules that, in normal conditions, bind cadherins, such as E-cad, to the actin cytoskeleton (Rosso et al., 2014), playing a crucial role in differentiation and cell proliferation (Angers & Moon, 2009; Nowicki, Sporny, & Duda-Szymańska, 2012).

Occludin and **ZO-1** are also epithelial markers that are important for the maintenance of cell-cell adhesion mediated by TJ, that usually become downregulated during EMT (Feldman, Mullin, & Ryan, 2005). The knockdown of **OCLN** in cancer, including breast cancer, leads to loss of adhesion between cells and an increase in the migratory and metastatic potential of cancer cells (Bottoni,

Isgrò, & Scatena, 2016; Odero-Marah, Hawsawi, Henderson, & Sweeney, 2018). This demonstrates that OCLN is essential for the correct function of TJ in epithelial cells (Ko et al., 2019; Martin, Mansel, & Jiang, 2010).

Finally, **EpCAM** is another transmembrane glycoprotein (Huang et al., 2018) that, during EMT, is usually lost (Gires & Stoecklein, 2014; Hyun et al., 2016; Pastushenko & Blanpain, 2019; H. Wang, Stoecklein, Lin, & Gires, 2017), which coincides with the increased expression of mesenchymal markers (Baumeister et al., 2018; Dongre & Weinberg, 2019; Gorges et al., 2012). However, in breast carcinomas, EpCAM is one of the main biomarkers used to isolate CTCs (Banyś-Paluchowski, Krawczyk, Meier-Stiegen, & Fehm, 2016; Bidard et al., 2014; Cabel et al., 2017), being considered a poor prognostic marker (Gires, Pan, Schinke, Canis, & Baeuerle, 2020).

1.3.2. Mesenchymal Markers

Mesenchymal cells, which have already undergone EMT, show a different shape and express a distinct set of markers in comparison to epithelial cells. Their morphology is more elongated and there is the expression of mesenchymal markers, such as **N-cadherin (CDH2/N-cad)**, **vimentin (VIM)**, **CD61/ITGB3 (ITGB3)** and **CD44**.

N-cad is a calcium-dependent transmembrane glycoprotein that mediates homo-and heterotypic cell-cell adhesion (Cao, Wang, & Leng, 2019). During EMT, it is assumed that there is a cadherin switch: as the expression of E-cad decreases and the expression of N-cad increases (Wheelock, Shintani, Maeda, Fukumoto, & Johnson, 2008). This increased expression of N-cad is crucial for migration and invasion of cancer cells, and, in addition, it is also a predictor of poor patients' prognosis (Luo et al., 2018; Masuda et al., 2020). N-cad upregulation occurs in several diseases, including breast cancer (Drivalos et al., 2016; Hui et al., 2013; Muramaki, Miyake, Terakawa, Kusuda, & Fujisawa, 2011; Saadatmand et al., 2013), and, in addition of being associated with the maintenance of the malignant tumor cell phenotype (Rezaei et al., 2012), it is also associated with the promotion of metastasis in several cancers (Hazan, Phillips, Qiao, Norton, & Aaronson, 2000).

Vimentin is an intermediate filament protein that acts as an important EMT marker (Z. Chen, Fang, & Ma, 2021; C. Y. Liu, Lin, Tang, & Wang, 2015). This protein is expressed in endothelial cells, fibroblasts, and lymphocytes; however it is almost undetected in most epithelial cells (Mendez, Kojima, & Goldman, 2010). *VIM* increased expression has been associated with several cancer cell lines and tissues, including epithelial tumors (Satelli & Li, 2011) and is associated with tumor invasion, migration and loss of differentiation (Gilles et al., 2003; Karihtala et al., 2013; Korsching et al., 2005). Due to this, *VIM* is considered a marker of the mesenchymal phenotype (Mandal et al., 2008).

CD61/ITGβ3 is a cell-matrix adhesion receptor on the cell surface that has low expression in normal epithelial tissues. However, during EMT, this protein is up-regulated in several cancers (Deep et al., 2014; Galliher & Schiemann, 2006; Lei et al., 2011). Thus, its presence is considered a poor prognosis indicator in numerous cancers, including breast cancer (Taherian, Li, Liu, & Haas, 2011). It is known that ITGβ3 can regulate angiogenesis and is involved in cancer metabolism, stemness, shaping the immune microenvironment and promoting EMT (Zhu et al., 2019). Furthermore, ITGβ3 silencing leads to inhibition of EMT and metastasis in breast cancer cells (Parvani, Gujrati, Mack, Schiemann, & Lu, 2015).

CD44 is a transmembrane glycoprotein, receptor of the hyaluronic acid, which is overexpressed in various cancers, including breast cancer (Brown et al., 2011; Eramo et al., 2008; Kryczek et al., 2012; Prince et al., 2007). It is associated with invasion, metastasis, and poor patient prognosis (Lakhtakia, Aljarrah, Furrukh, & Ganguly, 2017; Louderbough & Schroeder, 2011; Mani et al., 2008; H. Xu et al., 2016). In addition, it has been shown that CD44 knock down impairs cell invasion and migration in breast cancer cells (Prasad, Chaurasiya, Guilmain, & Andersson, 2016).

1.3.3. EMT Transcription Factors (TFs)

For several decades, it was believed that the overexpression of transcription factors (TFs) that would induce EMT was only associated to a late state of this process. However, several *in vivo* evidence has clearly demonstrated that EMT-

TFs can induce and promote early EMT, through the modulation of cell plasticity, migration, invasion, and even oncogenic transformation (Goossens, Vandamme, Van Vlierberghe, & Berx, 2017). It is known that EMT induction occurs independently of the cancer cell-type (Pal et al., 2019) and five EMT-TFs are currently well known: zinc finger E-box binding homebox 1/2 (ZEB1/2), snail family transcriptional repressor 1 (Snail), snail family transcriptional repressor 2 (Slug) and twist family bHLH transcriptional factor (Twist); and their overexpression not only contributes to the formation of a permissive tumor microenvironment, but is also correlated with tumor growth, progression and worsening of patient survival (Imani, Hosseinifard, Cheng, Wei, & Fu, 2016; Katsura et al., 2017).

ZEB2, also known as SiP1, is a transcription factor that promotes EMT and metastasis through its role as an E-cad suppressor and regulator of several genes codifying different molecules constituting epithelial cell-cell junctions (Comijn et al., 2001; He et al., 2017; Vandewalle et al., 2005). Moreover, this TF is able to upregulate some mesenchymal markers (Si et al., 2015; Vandewalle, Van Roy, & Berx, 2009). The high expression of this molecule is associated to rapid cell proliferation and poor patient survival in several tumors (Elloul et al., 2005; Mejlvang et al., 2007; Rosivatz et al., 2002). In contrast, its knockdown leads to inhibition of cell proliferation, migration and invasion (Browne, Sayan, & Tulchinsky, 2010; M. Hu et al., 2010; Sathyanarayanan, Chandrasekaran, & Karunakaran, 2017; Xia et al., 2010).

In addition, **ZEB1** is another TF that induces EMT through the repression of E-cad (Vannier, Mock, Brabletz, & Driever, 2013; H. T. Wu et al., 2020). It is also known that ZEB1 increased expression promotes chemo- and radio-resistance of tumor cells, which indicates that ZEB1, besides being involved in oncogenesis and metastasis, can also affect the overall survival of cancer patients (C. Wang et al., 2016; X. Zhang et al., 2018).

Snail is a zinc finger transcription factor whose expression is associated with migration, invasion, and metastasis of tumor cells (Assani & Zhou, 2019; Osorio, Farfán, Castellón, & Contreras, 2016; Y. Wang, Shi, Chai, Ying, & Zhou, 2013). This happens not only because Snail expression is able to inhibit the expression

of several proteins essential to cell-cell adhesion, namely E-cad, OCLN and ZO-1, but also because the knockdown of this TF inhibits tumor growth through the activation of an immune response (Blanco et al., 2002; Kudo-Saito, Shirako, Takeuchi, & Kawakami, 2009; Ohkubo & Ozawa, 2004; Peinado, Olmeda, & Cano, 2007). Moreover, it is also known that hypoxia can induce Snail expression within tumors, leading to EMT (Lundgren, Nordenskjöld, & Landberg, 2009).

Slug is another transcription factor that has been associated with EMT induction, since its expression not only leads to E-cad inhibition (Alves, Carneiro, Hoefler, & Becker, 2009; Bolós et al., 2003; M. Cheung et al., 2005), but also promotes cancer cell invasion and migration, as well as metastasis (Dai et al., 2021; A. Gu, Jie, Yao, Zhang, & Mingyan, 2017; Qian et al., 2013; Toiyama et al., 2013; Zhao et al., 2015).

Twist, a member of the basic helix-loop-helix transcription factor family, is a crucial transcription factor in EMT induction, as well as cell invasion and migration (Z. F. Chen & Behringer, 1995; Qin, Xu, He, Qin, & Xu, 2012; J. Yang et al., 2004). It is known that this TF is up-regulated, alongside with Snail and Slug, during EMT, and that plays a key role in Slug induction, further inhibiting E-markers and promoting EMT and metastasis (Casas et al., 2011; K. Lee & Nelson, 2012). Moreover, the ectopic expression of Twist 1 in breast cancer cells led to the acquisition of cancer stem-like properties and further induction of EMT (Cheng et al., 2007; J. Fu et al., 2011; Mani et al., 2008; J. Yang et al., 2004). This TF is not only involved in cancer invasion and metastasis, but can also promote angiogenesis through the up-regulation of M-markers (Che et al., 2011; Zhuo et al., 2015). Thus, all these results suggest that Twist plays a fundamental role in cancer progression, as it is involved in several processes (Y. Xu et al., 2017).

1.3.4. Hybrid Phenotype

Recently, new evidences have demonstrated the existence of intermediate or hybrid EMT phenotypes (hy-phen) (Pastushenko et al., 2018; Shibue & Weinberg, 2017). Importantly, cancer cells harboring a hybrid epithelial/mesenchymal phenotype were reported to play a crucial role in

metastasis, by promoting cellular plasticity, cancer stemness, collective migration and drug resistance (Garg, 2017; Jolly et al., 2015; Liao & Yang, 2020; Micalizzi et al., 2010).

However, the hy-phen is not yet fully characterized. Some authors have already divided it into two stable stages, the **early hybrid EMT state** and the **late hybrid EMT state**. In the first, cancer cells still have an epithelial and round-shaped morphology, but their cell-cell adhesion, although still present, has already decreased, with a lower expression of epithelial cell adhesion molecules, such as EpCAM and E-cadherin (E-cad). In addition, these cells start to express mesenchymal markers and transcription factors, such as vascular cell adhesion molecule 1 (VCAM1), Integrin $\beta 3$ (ITG $\beta 3$) and Integrin αV (ITG αV) (Aban et al., 2021; Pastushenko & Blanpain, 2019). Regarding the late hybrid EMT state, cells maintain the expression of markers found at the early state; however, they already almost lost cell-cell adhesion, as well as their round shape (Pastushenko & Blanpain, 2019), and the transition to a completely mesenchymal phenotype can be induced by constitutive ectopic expression of ZEB1 (Kröger et al., 2019).

1.3.4.1. Phenotypic Stability Factors (PSFs) of the hybrid EMT state

Phenotypic stability factors (PSFs) are molecules that allow cells to remain stable in a hybrid EMT phenotype (Biswas, Jolly, & Ghosh, 2019; Pasani, Sahoo, & Jolly, 2020). To this end, they can have different roles within the cell. While some maintain the epithelial phenotype, promoting cell-cell adhesion, being more involved in the maintenance of an early hybrid phenotype, others confer invasive and proliferative capacity, being associated to a late hybrid phenotype (Brabletz, Schuhwerk, Brabletz, & Stemmler, 2021; W. Chen et al., 2016; K. Saxena, Srikrishnan, Celia-Terrassa, & Jolly, 2020). However, little is known about the molecular mechanisms that promote the maintenance of the hybrid state.

In recent years, some hybrid PSFs have been proposed, such as **Ovol2 (OVOL2)**, **GRHL2 (GRHL2)**, **Integrin $\beta 4$ / ITG $\beta 4$ (ITGB4)**, **VCAM1 (VCAM1)**, **Δ Np63 (TP63)**, **AMPK (PRKAA2)**, **NRF2 (NFE2L2)**, **NUMB (NUMB)**, **ITG αV (ITGAV)**, **Smad3 (SMAD3)**, among few others (Hong et al., 2015; Jolly et al., 2017; Jolly et al., 2016; Sinha, Saha, Samanta, & Bishayee, 2020; Subbalakshmi

et al., 2020), once these are fundamental for maintaining the hy-phen, while preventing cells of undergoing the complete EMT process (Dang, Esparza, Maine, Westcott, & Pearson, 2015; Jolly et al., 2017; Watanabe et al., 2014).

Ovol2, a zinc finger TF, plays a key role in EMT (Watanabe et al., 2014), as its presence stabilizes the hybrid phenotype (Hong et al., 2015; Jolly et al., 2016; Watanabe et al., 2014) through the inhibition of transcription factors, such as Twist, that lead to the suppression of migration and invasion (Haensel et al., 2019; Z. H. Wang et al., 2017; Yilmaz & Christofori, 2009). Moreover, Ovol2 suppression leads to upregulation of EMT transcription factors and mesenchymal markers, which promotes the transition to the mesenchymal phenotype (Bai et al., 2018; H. Fu et al., 2016).

GRHL2 is another transcription factor deregulated in several cancers (Cieply et al., 2012). The knockdown of this molecule leads to the down-regulation of E-cad, and upregulation of ZEB1, promoting EMT, cell migration, invasion, and tumor progression (Cieply, Farris, Denvir, Ford, & Frisch, 2013; Faddaoui et al., 2017; Mlacki, Kikulska, Krzywinska, Pawlak, & Wilanowski, 2015; Werner et al., 2013; Xiang et al., 2012). Due to this, GRHL2 is considered a phenotypic stability factor of an early EMT, as its presence is crucial to maintain the hy-phen (Jolly et al., 2016).

Integrin $\beta 4$ (ITG $\beta 4$) is a heterodimeric transmembrane cell-matrix adhesion receptor overexpressed in several cancers, including breast cancer. Its overexpression is associated to the hy-phen (Kröger et al., 2019), as well as to an aggressive behavior of cancer cells, metastasis and poor patient survival (Carico et al., 1993; Guo & Giancotti, 2004; Hynes, 2002; Logsdon et al., 2003; S. Lu, Simin, Khan, & Mercurio, 2008; Wolf et al., 1990). In addition, ITG $\beta 4$ upregulation promotes EMT (Masugi et al., 2015) and tumor growth (X. L. Li et al., 2017).

VCAM1, a member of the immunoglobulin superfamily, is a cell adhesion protein that facilitates certain tumor-stromal interactions (Q. Chen & Massagué, 2012). Furthermore, VCAM1 is also directly related to tumor development and its expression is correlated with invasive and aggressive tumor characteristics, metastasis, angiogenesis, and patient poor prognosis (Q. Chen, Zhang, &

Massagué, 2011; Fukushi, Ono, Morikawa, Iwamoto, & Kuwano, 2000; Kong, Kim, Kim, Jang, & Lee, 2018; X. Lu et al., 2011; D. Zhang et al., 2020). In addition, during breast cancer progression, the serum levels of this molecule begin to increase (Byrne et al., 2000), which appears to be related to metastasis namely in brain, bone, and lung (Sharma et al., 2017).

Δ Np63 is one of the major isoforms of p63 which has demonstrated oncoprotein functions, such as invasion and metastasis, during cancer progression (Chakrabarti et al., 2014; Di Giacomo et al., 2017; Dohn, Zhang, & Chen, 2001; Patturajan et al., 2002; Ratovitski et al., 2001; G. Wu et al., 2003). However, the role of p63 in cancer is not clear. On one hand, Δ Np63 is a suppressor of EMT in prostate and bladder cancer (Tran et al., 2013; Tucci et al., 2012). Δ Np63- α and - β are EMT inhibitors. But Δ Np63- γ is an EMT promotor in some mammary epithelial cells (Lindsay, McDade, Pickard, McCloskey, & McCance, 2011). Furthermore, it is known that Δ Np63 loss or knockdown is correlated with the promotion of EMT, invasion, and metastasis (Giacobbe et al., 2016; Koga et al., 2003; Urist et al., 2002) and, additionally, in breast tumors, Δ Np63 is a key factor that is able to regulate tumor stemness and growth (Chakrabarti et al., 2014; Memmi et al., 2015).

Adenosine monophosphate-activated protein kinase (AMPK) has been related to various physiological processes, such as autophagy, cell division and growth, and maintenance of epithelial cell polarity (Jones et al., 2005; Liang et al., 2007; Xiang, Saha, Wen, Ruderman, & Luo, 2004; H. Zhang, Singh, Talukder, & Kumar, 2006). Furthermore, this protein has been associated with cancer cell migration and invasion (M. B. Chen et al., 2011; Kim et al., 2011) and its activation occurs under stress conditions, such as hypoxia (Steinberg & Kemp, 2009). These results, together with its ability to inhibit apoptosis and promote tumor growth, make this protein to be considered to have pro-tumorigenic functions (Hindupur et al., 2014; Jeon & Hay, 2015; Kim et al., 2011; Ng et al., 2012; X. Wang, Pan, & Song, 2010). In addition, AMPK activation or up-regulation induces EMT, and its presence helps to maintain the hybrid phenotype, through the downregulation of E markers (M. Saxena et al., 2018).

NRF2, also known as nuclear factor (erythroid-derived-2)-like 2 (NFE2L2), is a transcription factor that plays a dual role in cancer. Thus, NRF2 can act as an oncogene or as a tumor suppressor molecule (Menegon, Columbano, & Giordano, 2016; S. Wu, Lu, & Bai, 2019). In the past, this TF was believed to activate the cellular antioxidant response, preventing the death of healthy/normal cells (Motohashi & Yamamoto, 2004). However, new evidence has been showing that NRF2 also plays an important role on the survival of cancer cells, leading to the progression of the disease (Lau, Villeneuve, Sun, Wong, & Zhang, 2008; X. J. Wang et al., 2008). Furthermore, activation of this TF in several cancers induces the expression of pro-survival genes, prevents apoptosis, promotes cell proliferation, and enhances self-renewal capacity (S. Wu et al., 2019). In addition, NRF2 is also considered a PSF as it can prevent the complete progression of the EMT, stabilizing the *hy-phen* (Bocci et al., 2019).

NUMB is a phosphotyrosine-binding domain (PTB) protein involved in EMT and with a key role in migration, endocytosis, cell fate and cell polarity (Gulino, Di Marcotullio, & Screpanti, 2010; Nishimura & Kaibuchi, 2007; Uemura, Shepherd, Ackerman, Jan, & Jan, 1989; Z. Wang, Sandiford, Wu, & Li, 2009). The loss of NUMB protein expression has been associated with several types of cancer, including breast cancer, and with metastasis formation (Pece et al., 2004; Rennstam et al., 2010). Moreover, it has been shown that NUMB can negatively regulate EMT, in breast cancer cells, by modulating β -catenin expression (X. B. Hu, Ouyang, He, & Xia, 2019; J. Zhang et al., 2016). For all these reasons, NUMB is considered a tumor suppressor of cancer progression (Pece, Confalonieri, P, & Di Fiore, 2011; Tosoni et al., 2017), as well as a PSF, as its presence prevents the complete transition to the mesenchymal phenotype (Bocci et al., 2017).

Integrin α V (ITG α V) is a transmembrane glycoprotein involved in cell-matrix adhesion through the link between the ECM and the actin cytoskeleton of cells (Martino, Perestrelo, Vínarský, Pagliari, & Forte, 2018). ITG α V overexpression has been associated with several tumors, including breast tumors (Q. Chen et al., 2008), and is correlated with patient's poor prognosis (Niu & Li, 2017). In addition, the high expression of this glycoprotein promotes EMT, cell proliferation, migration and invasion, metastasis and chemoresistance (Cheuk et al., 2020; McNeill, Vulesevic, Ostojic, Ruel, & Suuronen, 2015; H. Wang et al., 2019). In

contrast, ITGαV-negative tumors, have an increased expression in cell adhesion molecules, such as E-cad (Kemper et al., 2021). Thus, ITGαV is considered a poor prognostic factor (Loeser et al., 2020) and a marker of late hybrid EMT (Pastushenko & Blanpain, 2019).

Smad3 is a transcription factor enriched in late EMT (Pastushenko & Blanpain, 2019) and its expression induces the expression of both E and M markers (C. Li et al., 2016). In addition, this TF can promote EMT and give cells' resistance to some cancer therapies (Shi, Zhang, Liu, Huang, & Yin, 2019). However, high expression of Smad3 can also suppress metastasis and tumor progression (S. Lu et al., 2007; Y. Yang, Guan, Guo, & Han, 2018).

1.4. P-cadherin

1.4.1. P-cadherin molecular structure and biological function

P-cadherin (P-cad), also known as placental cadherin, was described for the first time in mouse placenta (Nose & Takeichi, 1986; Shimoyama et al., 1989). However, despite its name, there is no expression of P-cad in the human placenta. P-cad is a cell-cell adhesion glycoprotein, encoded by the *CDH3* gene. Its structure is quite similar to the structure of classical cadherins (E-cad and N-cad), being divided into three different domains: extracellular, transmembrane and cytoplasmic (Nollet, Kools, & van Roy, 2000; Nose & Takeichi, 1986).

Regarding its biological function, P-cad performs homeostatic functions in many normal tissues (Ribeiro & Paredes, 2014) being, together with other classical cadherins, one of the main adhesive proteins at intercellular junctions (Leckband & Sivasankar, 2012). Moreover, in adult tissues, this glycoprotein is frequently co-expressed with E-cad, being mostly predominant in the basal layer of several epithelial tissues (Albergaria et al., 2011; Vieira & Paredes, 2015).

1.4.2. P-cadherin role in breast cancer: biology and prognosis

Due to P-cad crucial role in normal development, aberrant expression of this protein has been frequently linked to many tumors (Behrens, 1999; Palacios et

al., 1995; Paredes et al., 2007; Paredes, Milanezi, Viegas, Amendoeira, & Schmitt, 2002; Wood & Leong, 2003). In several cancer models, P-cad overexpression induces tumor cell motility and promotes migration (L. W. Cheung, Leung, & Wong, 2010; Mandeville et al., 2008; Ribeiro et al., 2010; Taniuchi et al., 2005). Furthermore, in addition to its high correlation with invasion in several tissues (N. Liu et al., 2012; Plutoni, Bazellières, & Gauthier-Rouvière, 2016), the presence of this cadherin has been considered a marker of poor prognosis in breast cancer (Paredes et al., 2005; Turashvili et al., 2011), since P-cad expressing tumors are associated with short-term locoregional relapse-free interval, as well as overall survival (Gamallo et al., 2001; Peralta Soler, Knudsen, Salazar, Han, & Keshgegian, 1999). Additionally, P-cadherin overexpression is also associated with clinicopathological features, namely molecular subtype and molecular phenotype, high histological grade and lymph node metastasis (Qi et al., 2020).

However, P-cadherin also has a dual role in cancer depending on the molecular context and the tumor cell model used (Vieira & Paredes, 2015). Opposite effects for P-cadherin have already been reported in some tumor models, namely bladder, prostate and colon carcinomas. Thus, while some studies show that P-cad has a tumor suppressor role (Jarrard et al., 1997; Mandeville et al., 2008; Van Marck, Stove, Jacobs, Van den Eynden, & Bracke, 2011), others relate the P-cadherin expression to a more aggressive behavior (Arenas, Romo, Royuela, Fraile, & Paniagua, 2000; Van Marck et al., 2011; P. Wang et al., 2014).

After describing that P-cad overexpression promotes an aggressive behavior in breast tumors (Paredes et al., 2005), our group demonstrated for the first time that P-cad induces stem-like properties to breast cancer cells since this adhesion protein was directly associated with the expression of the cancer stem cell markers CD44, CD49f and aldehyde dehydrogenase 1 (ALDH1), which are stem cell markers (Vieira et al., 2012).

P-cad role in breast cancer cells was also shown to be dependent on the expression of E-cad. It was observed that the co-expression of these two classical cadherins, simultaneously, increases tumor growth *in vivo* and collective

cell invasion; however, in the same cancer models, P-cad was able to suppress the invasion and tumorigenesis in the absence of E-cad (Ribeiro et al., 2013).

Furthermore, P-cad inhibition was reported to lead to a significant reduction in cancer cells adhesion to the basement membrane substrate laminin, as well as to a major decrease in the expression of a heterodimer, crucial for invasive capacity: the laminin receptor $\alpha 6\beta 4$ integrin. This demonstrates that P-cad mediates breast cancer cells adhesion to ECM, as well as collective migration (Vieira et al., 2014).

Additionally, our group demonstrated that P-cad overexpression in breast cancer cells induces alterations in their biomechanical properties, namely the increase in their migratory and invasive capacity. These changes depend on the SFK pathway activation, which is mediated by P-cad expression (Ribeiro et al., 2016). Therefore, inhibitors of this pathway, such as dasatinib, could not only repress P-cad signaling effects, as well as its physical and mechanical impacts. Based on this evidence, we further evaluated how dasatinib treatment could affect tumor aggressiveness. And here, we found that P-cad overexpression in breast cancer cells is associated with SRC activation, and its suppression decreases, *in vitro*, cell migration and invasion, as well as the ability to form mammospheres. *In vivo*, dasatinib treatment decreases tumorigenic and metastatic capacity, increasing mice overall survival (Ribeiro et al., 2018).

Finally, our group has recently shown that breast cancer cell populations expressing P-cad are more probable to present increased glycolysis and to survive to metabolic-driven changes, since P-cad expression is correlated with glycolytic, hypoxic and acidosis markers (Sousa, Pereira, & Paredes, 2019; Sousa et al., 2014). Our group successfully demonstrated that P-cad expression is early promoted by EMT-inducers, such as hypoxia, and that it can drive *anoikis*-resistance of matrix-detached breast cancer cells (Sousa et al., 2020).

Due to all these findings regarding the role of P-cadherin expression in cancer, this has led to the development of anti-P-cadherin therapeutic approaches, being some of them already in clinical trials (Root et al., 2016; Sheng et al., 2021; C. C. Zhang et al., 2010).

1.4.3. P-cadherin and EMT

The results obtained so far by our group, as well as by other authors, led us to hypothesize that P-cadherin could be a promising marker of the hybrid or intermediate EMT phenotype (Acloque, Ocaña, Abad, Stern, & Nieto, 2017; Idoux-Gillet et al., 2018; Y. J. Lee, Bernstock, Klimanis, & Hallenbeck, 2018; Ribeiro & Paredes, 2014). The rationale for this hypothesis relies on the fact that P-cadherin expression disturbs epithelial cell–cell adhesion and promotes the acquisition of a more undifferentiated cell phenotype, acquiring these cells an intermediate phenotypic state between epithelial and mesenchymal morphology (Paredes et al., 2005; Ribeiro et al., 2013). Additionally, P-cadherin-overexpressing cells show increased therapy resistance, stem cell properties, and a more aggressive and invasive behavior (Ribeiro & Paredes, 2014). Finally, P-cadherin expression is a factor of poor prognosis in breast cancer, being associated to tumors with high metastatic potential. Thus, all these characteristics points to a transient EMT state, with cancer cells harboring an increased plasticity and metastability.

This premise seems to be corroborated by preliminary data that we have recently produced, where a three-protein EMT signature has been applied to a large series of invasive breast carcinomas, using the expression of two classical EMT markers, E-cadherin and vimentin, combined with P-cadherin expression. Our results show that P-cadherin identifies an intermediate state between the epithelial and the mesenchymal phenotypes, associated to a poor prognosis in breast cancer patients, but also with the expression of breast cancer stem cell markers (Figure 4). Although this data needs further validation, both in independent breast cancer series and by *in vitro* and *in vivo* functional assays, our future aim is to definitely prove the crucial role of P-cadherin in identifying the EMT intermediate or hybrid phenotype. Proving this hypothesis, we will clarify the role of P-cadherin in the EMT process, identifying it as a putative EMT marker and supporting the use of anti-P-cadherin therapeutics to metastatic breast cancer.

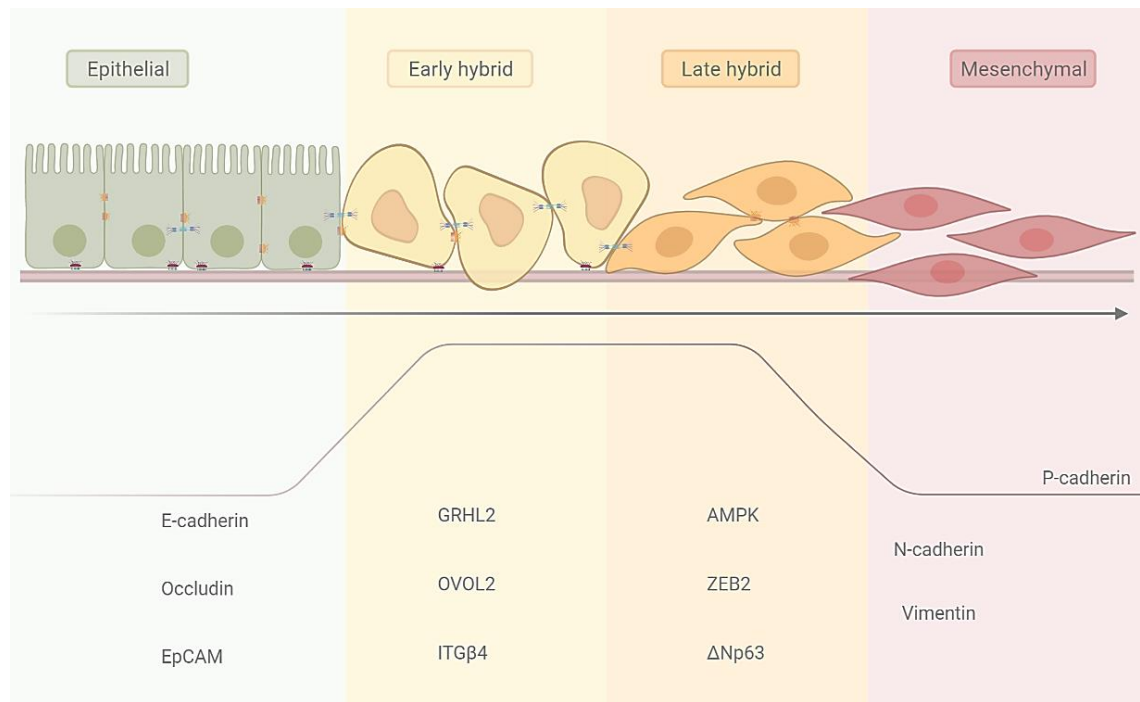


Figure 4. Can P-cadherin expression act as a PSF or as a biomarker of a stable hybrid E/M phenotype (hy-phen) in breast cancer cells? [Image created in Biorender].

2. General Objectives

Despite the role of P-cadherin expression in breast cancer patient's poor prognosis and the *in vitro* evidence showing that P-cad-mediated cell-cell adhesion enables cancer cells to be enriched in stem-like properties, to be *anoikis*-resistant and to be invasive in a collective manner, little is known about the role of this glycoprotein during the EMT process.

Thus, based on our previous data, the principal goal of this study was to investigate if P-cadherin expression can act as a PSF or even as a biomarker of a stable hybrid E/M phenotype (*hy-phen*) in breast cancer cells, operating as a lever of phenotypic cell plasticity that drives metastatic aggressiveness. In order to achieve this main goal, two specific aims have been proposed:

2.1 To assess the correlation between P-cadherin expression and the expression of EMT markers in a panel of breast cancer cell lines

The Cancer Cell Line Encyclopedia (CCLE) online database has been used to study the correlation between mRNA expression of *CDH3* (P-cadherin codifying gene) and the expression of several transcripts associated to EMT in a large panel of 57 breast cancer cell lines.

2.2 To investigate if P-cadherin expression regulates EMT biomarkers, including PSFs associated to a stable hybrid E/M phenotype

In an attempt to understand if P-cadherin expression would play a crucial role in maintaining an EMT *hy-phen*, we decided to genetically manipulate its expression, by overexpression or silencing, in three breast cancer cell lines from distinct molecular subtypes. With this approach, our aim was to study how alterations in the expression of this cell-cell adhesion molecule would affect the expression of EMT biomarkers.

3. Materials and Methods

3.1. Bioinformatic analysis

The Cancer Cell Line Encyclopedia (CCLE) online database (<https://sites.broadinstitute.org/ccle/>) was used to extract the data related with the expression levels of *CDH3* mRNA from a series of 57 breast cancer cell lines, as well as the expression of transcripts of biomarkers already associated to EMT, namely *CDH1*, *EPCAM*, *OVOL2*, *GRHL2*, *OCLN*, *ITGB4*, *CDH2*, *ITGB3*, *ZEB2*, *ZEB1*, *TP63*, *VCAM1*, *VIM*, *TWIST1*, *PRKAA2*, *SNAI2*, *SNAI1*, *CD44*, *CTNNB1*, *NFE2L2*, *ITGAV*, *NUMB*, *TJP1*, and *SMAD3* (Table S 1). We also organized the information according with the molecular subtype of each breast cancer cell line, as already described (Neve et al., 2006) (Table S 2). Finally, all data has been analyzed by the GraphPad Prism v8 software and the correlation between *CDH3* expression and EMT biomarkers was disclosed.

3.2. Cell Culture

Human breast cancer cell lines were obtained as follows: 1) MCF-7/AZ was provided by Prof. Marc Mareel (Ghent University, Belgium), 2) BT-20 was obtained from American Type Culture Collection (HTB19, ATCC, Manassas, VA, USA), 3) SUM149 was gently provided by Dr. Stephen Ethier (University of Michigan, USA). The MCF-7/AZ cell line was retrovirally transduced to stable encode EGFP (LZRS-IRES-EGFP plasmid, MCF-7/AZ.mock cell line) or both P-cadherin and EGFP (LZRS-P-cad-IRES-EGFP plasmid, MCF-7/AZ.P-cad cell line), as previously described (Paredes et al., 2004).

Cells were cultured in the following media: DMEM (Gibco) for BT-20; and 50% (v/v) DMEM (Gibco) + 50% (v/v) Ham F-12 (Gibco), supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Cytivia), 100 IU/mL penicillin and 100 mg/mL streptomycin (Invitrogen) for MCF-7/AZ; or supplemented with 5% (v/v) heat-inactivated FBS, 50 IU/mL penicillin and 50 mg/mL streptomycin, for SUM149. In addition, SUM149 medium was supplemented with 5 µg/mL of insulin and 1 µg/mL of hydrocortisone (Merck). All cell lines were routinely maintained at 37°C with 5% CO₂.

3.3. siRNA transfection

Gene silencing was carried out in BT-20 and SUM149 cell lines using a certified small interfering RNA (siRNA), from Qiagen, specific for *CDH3* (50 nM, Hs_CDH3_6, GW Validated siRNA, Qiagen), with the subsequent target sequence 5' AAGCCTCTTACCTGCCGTAAA 3'. Transfections were performed using Lipofectamine 2000 (Invitrogen) and according to manufacturer's recommended techniques. In addition, a scrambled siRNA targeting sequence 5' AATTCTCCGAACGTGTCACGT 3', with no homology to any gene, was used as a negative control (Qiagen) to validate the specificity of the results acquired. 48 hours after cell transfection, gene inhibition was evaluated by Western Blot.

3.4. Protein extraction and Western Blot

Cells were first washed twice with PBS and after were allowed to lyse in 500µL of catenin lysis buffer [1% (v/V) Triton X- 100 and 1% (v/V) NP-40 (Merck) in PBS] with 1:7 protease inhibitor cocktail (Roche Diagnostics) and with 1:100 phosphatase inhibitor cocktail 3 (Merck) for 10 min, at 4°C. Cells were then scraped and lysates were submitted to vortex 3 times and later centrifugated at 4°C, 10 min, at 14000 rpm. The supernatants were collected and protein concentration was measured using the Lowry assay (Bio-Rad DC Protein Assay kit, Bio-Rad). After, proteins were dissolved in sample buffer [Laemmli with 5% (v/V) 2-β-mercaptoethanol and 5% (v/V) bromophenol blue] and boiled for 5 min at 95 °C. Finally, samples were separated by 10% SDS-PAGE and proteins were transferred into nitrocellulose membranes (Amersham Hybond ECL, Amersham biosciences) at 100V, for approximately 120 min. After that, membranes were blocked for 1 hour, at RT, with 5% (w/V) non-fat dry milk, or 5% BSA, in PBS 1x containing 0,5% (v/V) Tween-20 (PBS-T). For immunostaining, membranes were incubated overnight at 4 °C with the primary antibodies (Table S 3). In the next day, membranes were washed five times, for 5min, with PBS-T, and then were incubated with anti-mouse or anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibodies (dilution 1:2000) for 1 h. After incubation, membranes were again washed with PBS-T. Proteins were later detected using Enhanced Chemiluminescence (ECL) reagent (Amersham) as a substrate and

blots were exposed to an autoradiographic film. Finally, the bands obtained were analyzed and quantified using Quantity One software (Bio-Rad). Each immunoblot was performed at least three times and the images presented are the most representative of the results obtained.

3.5. Statistical analysis

Results are shown as the Mean $\pm$ SD of three or more independent biological replicates. Statistical analyses were performed by GraphPad Prism v8 software, *Unpaired t test*, and *p*-values lower than 0,05 were considered statistically significant.

4.Results

4.1 Bioinformatic screening of *CDH3* expression and relevant EMT markers in breast cancer cell lines

The main objective of this first task was to search for available data and evidence that could support our hypothesis. Thus, the association between P-cad expression and different EMT markers was studied in a series of breast cancer cells, in order to validate whether P-cad could be a putative PSF or biomarker of the early EMT *hy-phen*. For this, the CCLE online database was examined and bioinformatic analyses were performed as predictive tools.

4.1.1. Correlation between relevant EMT genes and *CDH3*

As referred in the Introduction section, a total of 24 EMT biomarkers were identified from the literature, being some associated to epithelial or mesenchymal phenotypes, as well as to putative hybrid EMT phenotypes. Using the CCLE online database, we extracted the data related to the mRNA expression of these 24 genes in the available series of 57 breast cancer cell lines (Figure 5).

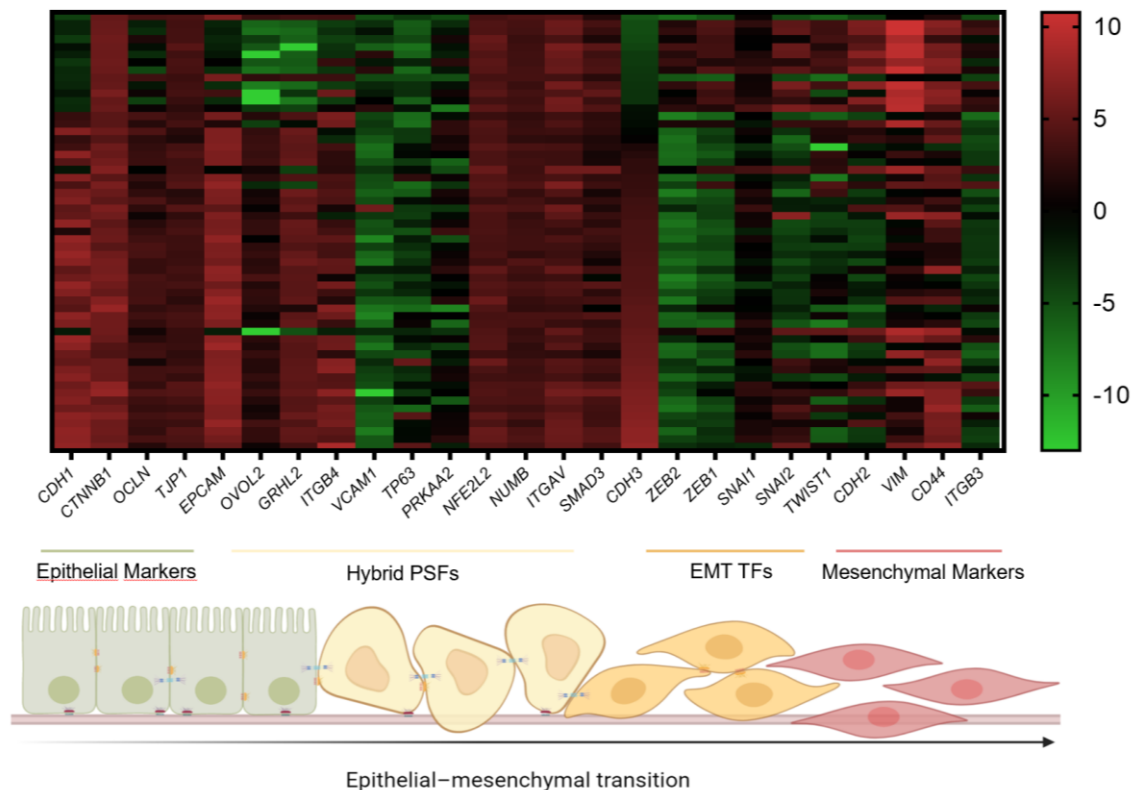


Figure 5. mRNA expression heatmap of the different EMT genes for all breast cancer cell lines.

Since our intention was to analyze which EMT transcripts would have a significant correlation with *CDH3* expression, we have used bioinformatic and statistic tools to find it. Interestingly, 13 out 24 (54,2%) were significantly correlated with *CDH3* expression. In detail, and as expected, a positive association was found with markers associated to epithelial cells, such as *CDH1* ($p<0,0001$), *EpCAM* ($p<0,0001$), *OCN* ($p<0,0001$), *ITGB4* ($p<0,0001$), *GRHL2* ($p<0,0001$), *OVOL2* ($p<0,0001$), and *TP63* ($p<0,0001$). Accordingly, an inverse correlation was observed between *CDH3* expression and mesenchymal makers, such as *CDH2* ($p=0,0004$), *VCAM1* ($p=0,0003$), *VIM* ($p=0,0002$), *ITGB3* ($p<0,0271$), *ZEB1* ($p<0,0001$) and *ZEB2* ($p<0,0001$) (Figure 6). Although not significant, there was also a trend for an inverse association with other EMT TFs, such as *TWIST1*, *SNAI1* and *SNAI2*, and for a direct association with *CTNNB1*. No correlation was found with the expression of *TJP1*, *CD44*, *SMAD3*, *NUMB*, *PRKAA2*, *ITGAV* and *NFE2L2* (Figure S 1).

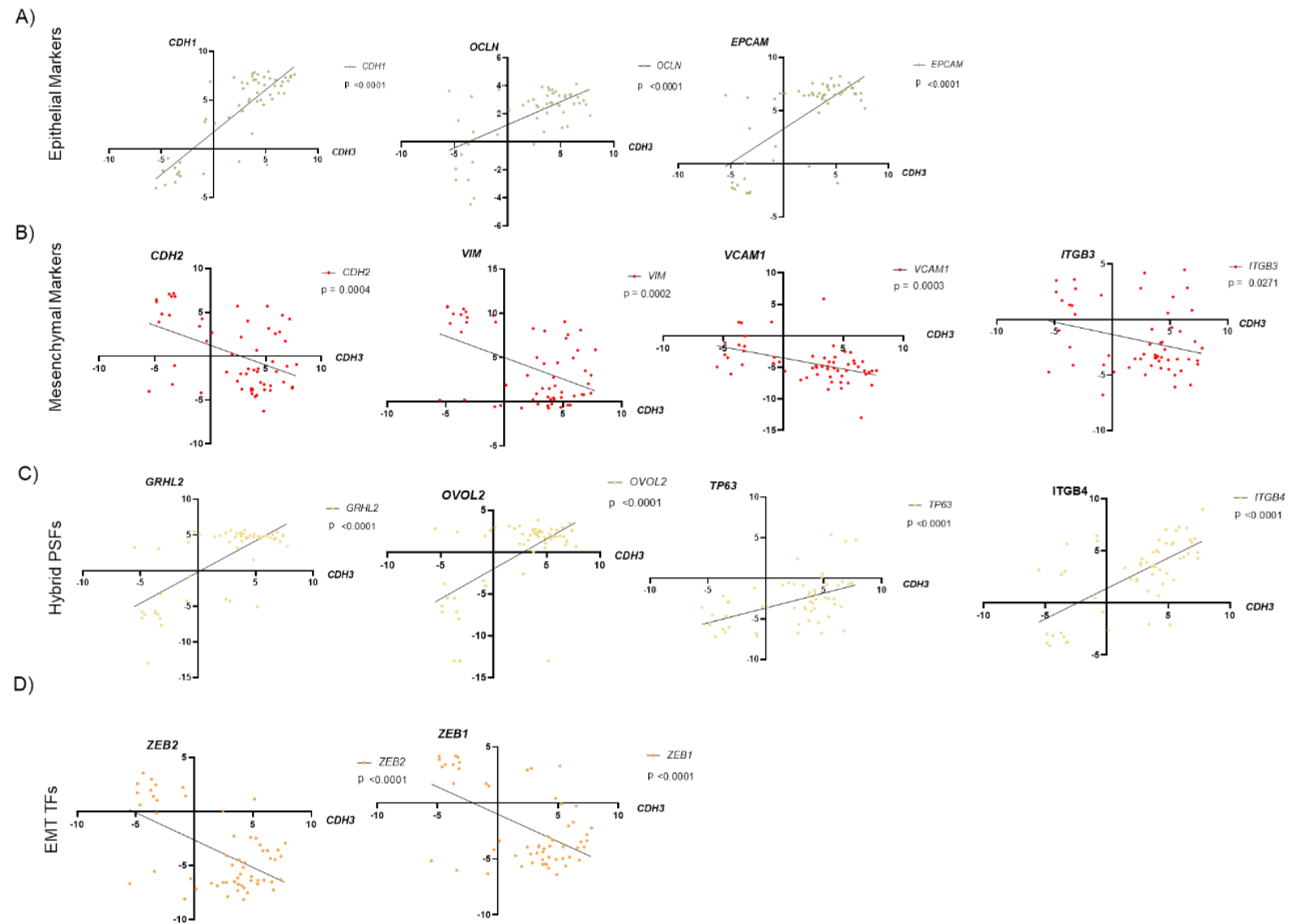


Figure 6. Correlation between relevant EMT genes and CDH3. A) Correlation between Epithelial markers, such as CDH1, OCLN and EpCAM, and CDH3. B) Correlation between Mesenchymal markers, such as CDH2, VIM, VCAM1 and ITGB3, and CDH3. C) Correlation between Hybrid PSFs, such as GRHL2, OVOL2, TP63 and ITGB4, and CDH3. D) Correlation between relevant EMT TFs, namely ZEB2 and ZEB1, and CDH3.

4.1.2. Expression of *CDH3* and EMT biomarkers within breast cancer cell lines' molecular subtypes

As previously described by Neve and the collaborators breast cancer cell lines can also be classified in different molecular subtypes, using the same methodology applied to classify invasive ductal breast carcinomas. Thus, according with their transcriptomic profile, breast cancer cell lines were clustered into three different subtypes: Luminal, Basal A, and Basal B. Interestingly, the Luminal subtype included mainly cancer cell lines that rely on estrogen signaling as Luminal tumors, being constituted by cells more differentiated and less invasive, and expressing several epithelial markers involved in cell-cell junctions. In contrast, Basal cell lines were mainly triple-negative and less differentiated, but where two specific subtypes (A and B) were able to be identified. Basal A subtype included cancer cell lines displaying a basal-like profile (such as basal-like carcinomas), expressing either epithelial and mesenchymal markers (hybrid phenotype) and which are able to invade in a collective manner. In addition, Basal B cell lines are the ones less differentiated, with increased invasive capacity and with a more mesenchymal appearance (Neve et al., 2006).

By applying this classification to the series of breast cancer cell lines included in the CCLE database, we graphically analyzed the expression of *CDH3* and of the 24 EMT biomarkers. We were able to confirm that there is a different gene expression distribution for each biomarker dependent on the breast cancer subtype (Figure 7).

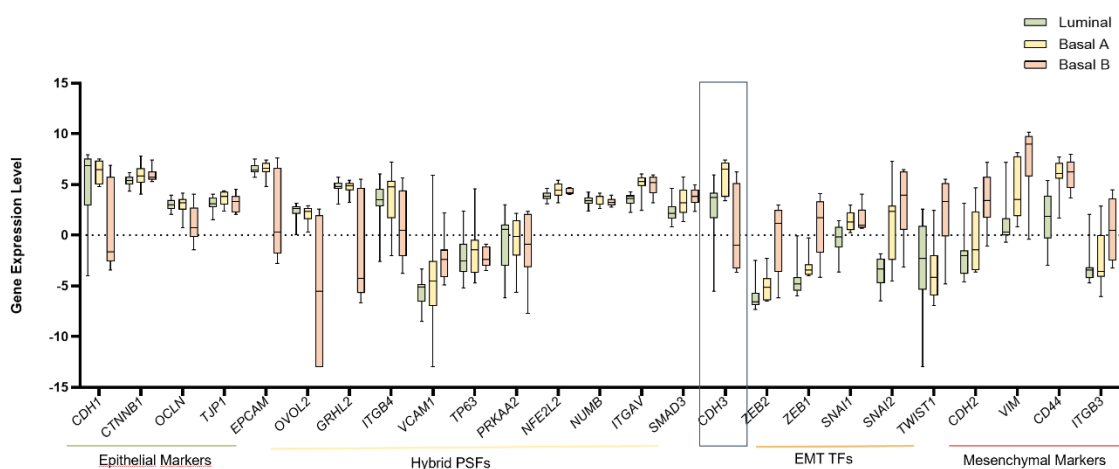


Figure 7. Gene expression level of *CDH3* and EMT biomarkers according to the breast cancer subtype.

As expected, we could observe that epithelial biomarkers were mainly enriched in Luminal and Basal A cell lines, such as *CDH1*/E-cadherin, *OCLN* and *EPCAM*, whereas mesenchymal associated markers were highly expressed in Basal B cell lines, namely *CDH2*/N-cadherin, *VIM*, *CD44* and *ITGB3*. However, in contrast with what was anticipated, the expression of *CTNNB1* and *TJP1* was mainly enriched in all cell lines. In addition, for transcription factors, *ZEB2*, *ZEB1*, *SNAI1*, *SNAI2* and *TWIST*, its expression was also high in Basal B cell lines.

Concerning gene expression of putative biomarkers of an early hybrid EMT phenotype, we could find that *OVOL2*, *GRHL2* and *ITGB4* are highly expressed in Luminal and Basal A cell lines, whereas their expression is more dispersed within Basal B cell lines. However, the expression of *VCAM1*, *TP63*, *PRKAA2*, *NFE2L2*, *NUMB*, *ITGAV* and *SMAD3* was not clearly associated with any subtype, being their expression enriched in all cell lines.

Interestingly, when we analyzed *CDH3* gene expression according to the molecular subtype of the distinct breast cancer cells lines, it was the only transcript that was actually highly enriched in Basal A cell lines in comparison to Luminal or Basal B cells, in accordance with our hypothesis that *CDH3*/P-cadherin can be an important biomarker of cells harboring an EMT hybrid phenotype.

4.2 Functional analysis to investigate if P-cadherin expression regulates EMT biomarkers

After bioinformatic analysis, where we found a significant association between *CDH3*/P-cadherin gene expression and the expression of different EMT transcripts, as well as its enrichment in Basal A cell lines that harbor epithelial and mesenchymal characteristics, we aimed to study if those correlations were functionally relevant *in vitro*.

In order to do that, we selected three breast cancer cells lines: MCF-7/AZ, BT-20 and SUM149, each of them representing a different breast cancer molecular subtype.

MCF-7/AZ is a Luminal cell line, isolated from a breast cancer metastasis, ER positive and HER2 negative. The expression levels of P-cadherin are low in this cell line, when compared with the expression levels of E-cadherin. In contrast, BT20 and SUM 149 cell lines are basal-like, being both highly positive for *CDH1* and *CDH3* expression. BT20 cells were classified as Basal A and were isolated from a breast ductal carcinoma ER and HER2 negative, whereas SUM149 were isolated from an Inflammatory Breast Cancer, also ER and HER2 negative, but were classified as Basal B by transcriptomics besides their expression of cell-cell adhesion molecules (Figure 8).

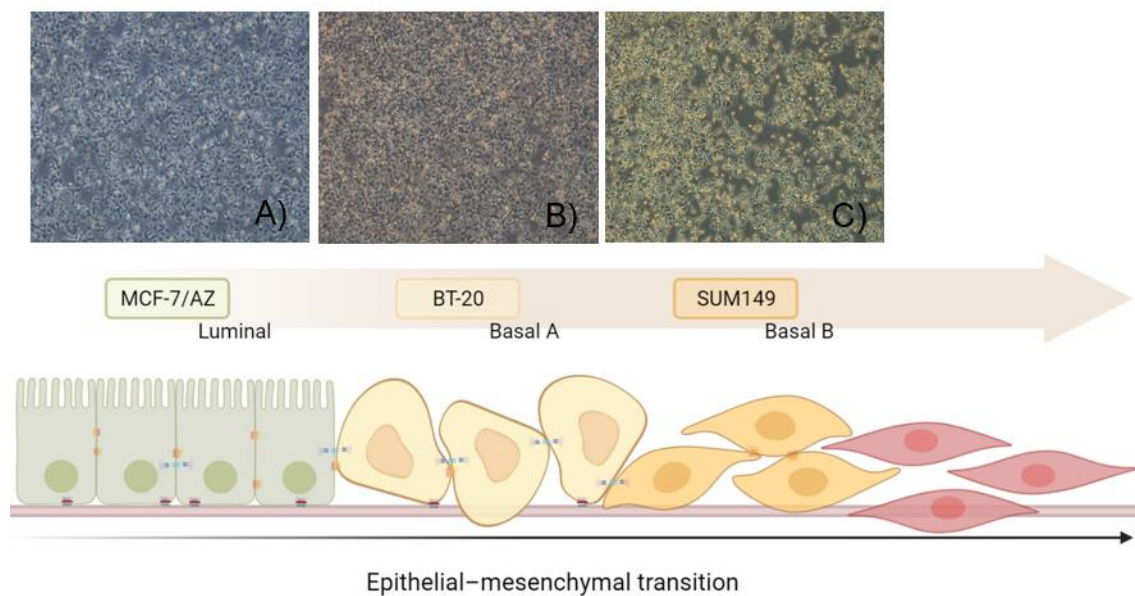


Figure 8. Breast cancer cell lines. A) MCF-7/AZ cells. B) BT-20 cells. C) SUM149 cells. Schematic representation of cell lines. Organization of cell lines according to their characteristics and the subtype of breast cancer to which they are associated. [Image created in Biorender].

In order to test if P-cadherin expression could play a role in the expression of different EMT biomarkers that were found to be significantly associated to its expression, we manipulated P-cadherin protein levels in these 3 cell lines, either by overexpressing *CDH3* cDNA in MCF-7/AZ, or by silencing *CDH3* mRNA in BT-20 and SUM149 cell lines. We have selected these conditions, since we have previously described in these cancer cell models that P-cadherin expression is related with increased tumorigenic and invasion capacity, as well as stem cell

properties and *anoikis*-resistance (Paredes et al., 2005; Paredes et al., 2008; Ribeiro & Paredes, 2014; Sousa et al., 2020)

Thus, we aimed to study, in these same cell lines, if P-cadherin expression would also interfere with the expression of EMT biomarkers. From the 13 markers significantly associated with *CDH3* expression in bioinformatic analysis, we were able to get and optimize the antibodies only for 9 of them, namely E-cadherin, occludin, EpCAM, N-cadherin, vimentin, ZEB2, ITG β 4, GRHL2 and Δ Np63. Although not significantly associated with *CDH3* in bioinformatic analysis, we have also studied the expression of AMPK and its functional phosphorylated form (pAMPK).

4.2.1 MCF-7/AZ – a Luminal breast cancer cell line

In order to evaluate how P-cad expression would influence the expression of EMT markers, we compared two conditions: MCF-7/AZ.mock and MCF-7/AZ.P-cad, which overexpress P-cad cDNA. We have previously shown that overexpression of P-cad in MCF-7/AZ cells induces cell motility, collective migration and invasion, as well as *anoikis*-resistance. Moreover, as already mentioned, P-cad expression is considered a marker of poor prognosis in breast cancer (Mandeville et al., 2008; Paredes et al., 2005; Ribeiro et al., 2010; Sousa et al., 2020; Taniuchi et al., 2005; Turashvili et al., 2011). Therefore, we assumed that P-cad overexpression would possibly induce the progression in EMT.

As first step, P-cad expression was analyzed, by western blot, to prove that, in fact, there was an overexpression of this protein ($1,594 \pm 0,410$) in MCF-7/AZ P-cad cells when compared to control, MCF-7/AZ Mock (Figure 9).

Concerning the expression of epithelial markers, such as E-cad, OCLN and EpCAM, the overexpression of P-cad did not affect their levels in a significant way (Figure 9).

Regarding mesenchymal markers, P-cad overexpression did not affect N-cad expression, which was already extremely low in this cell line. However, regarding VIM, which was unexpectedly strong in these cells, we could observe a significant

decrease in its expression ($1 > 0,687 \pm 0,192$) when P-cad was overexpressed (Figure 9).

In the case of the EMT transcription factor ZEB2, we could see that P-cad overexpression led to a significant increase of its expression ($1 < 1,347 \pm 0,311$) (Figure 9).

Finally, regarding putative PSFs associated to hybrid EMT states, we analyzed the expression of GRHL2, Integrin $\beta 4$, Δ Np63 and AMPK. We observed that P-cad overexpression did not affect Δ Np63 expression (Figure 9). However, for GRHL2, Integrin $\beta 4$ and tAMPK, P-cad overexpression led to a significant decrease on their expression ($1 > 0,542 \pm 0,270$, GRHL2; $1 > 0,611 \pm 0,294$, ITG $\beta 4$; $1 > 0,784 \pm 0,118$, tAMPK) (Figure 9). In addition, we have also analyzed the phosphorylated levels of AMPK at Thr172, pAMPK, since in this form this enzyme is more active (Willows et al., 2017), and we verified that P-cad overexpression also leads to a significant decrease on its phospho expression ($1 > 0,727 \pm 0,235$) (Figure 9).

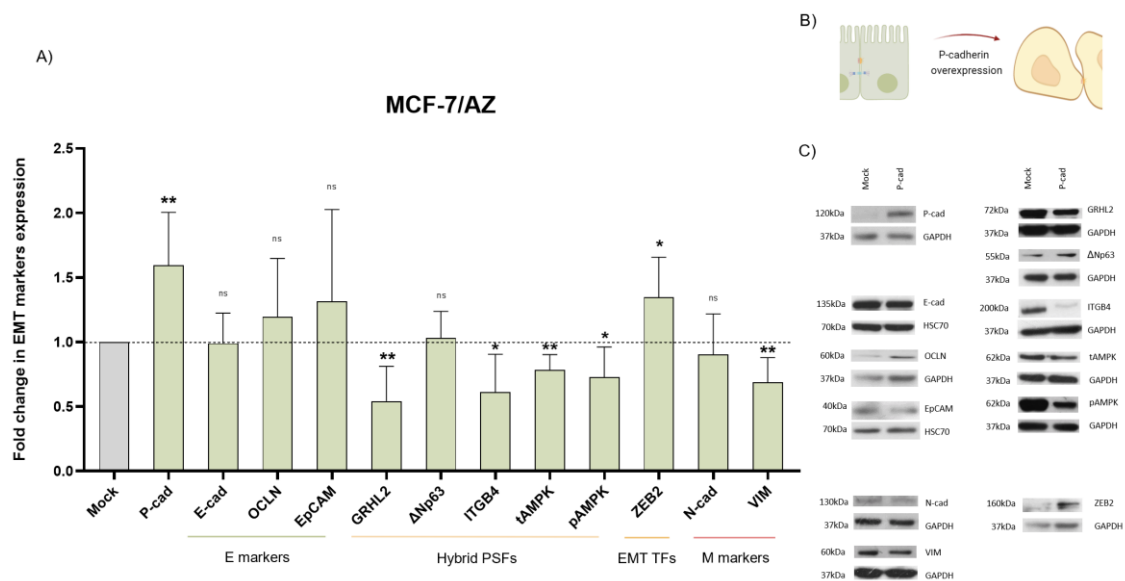


Figure 9. Evaluation of E and M markers, EMT TFs and hybrid PSFs expression in MCF-7/AZ cell line upon P-cad overexpression. A) Quantification of P-cadherin, E-cadherin, OCLN, EpCAM, GRHL2, GRHL2, Δ Np63, Integrin $\beta 4$, tAMPK, pAMPK, ZEB2, N-cadherin and Vimentin proteins; (Mean $\pm$ SD, N = 6-8-7-6-5-5-5-5-5-5-5-5, n $\geq$ 3, Unpaired t test); (*p < 0.0332, **p < 0.0021, ***p < 0.0002, ****p < 0.0001). B) Schematic representation of cellular alterations in P-cadherin-overexpressing cells. [Image created in Biorender]. C) Representative images of Western Blot.

In general, P-cad overexpression promotes EMT progression, but not a complete transition to the mesenchymal phenotype. Thus, P-cad regulates not only epithelial and mesenchymal markers, but also TFs and PSFs, so that cells acquire an early hybrid EMT state (Figure 10).

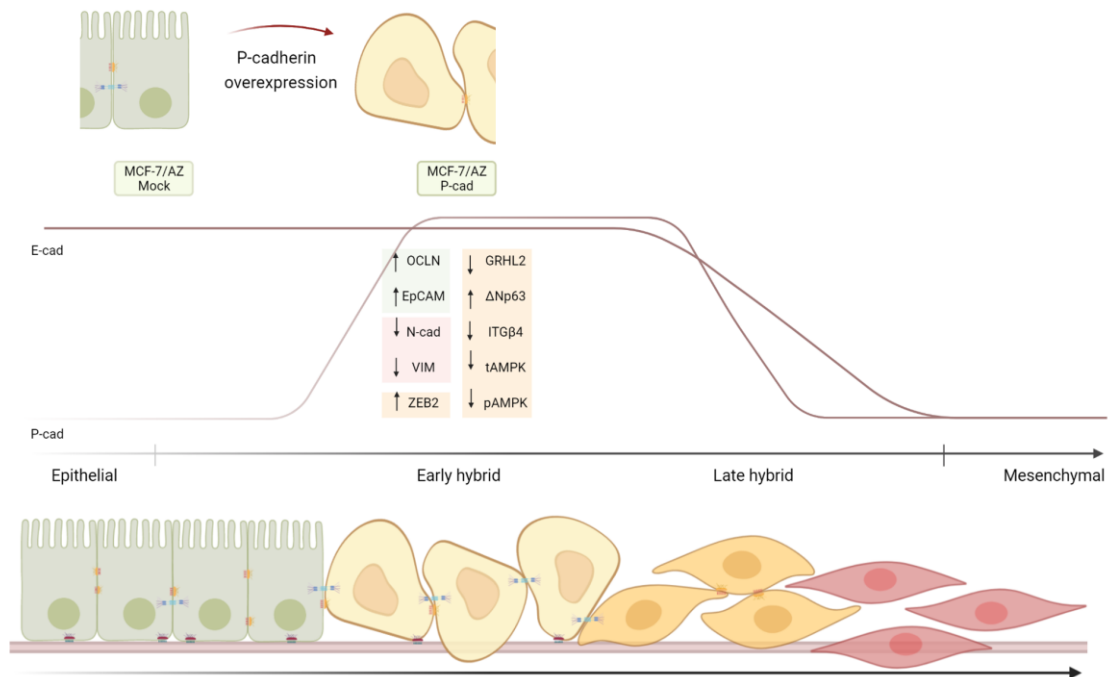


Figure 10. Schematic representation of the alteration in the expression of the different EMT markers with the overexpression of P-cadherin in the MCF-7/AZ cell line. [Image created in Biorender].

4.2.2 BT-20 – a Basal A breast cancer cell line

Regarding BT-20 cells, we have performed the silencing of *CDH3* mRNA, which codifies for P-cad expression. After, we analyzed how the expression of EMT markers would change. Based on previous studies, we estimated that P-cad silencing would induce the inhibition of EMT markers, leading cells to acquire a more epithelial-like phenotype (Ribeiro et al., 2013). Actually, we have previously described that P-cadherin silencing leads to a lower invasive capacity by BT-20 cells, which also become less tumorigenic and metastatic, as well as less resistant to *anoikis* and more responsive to chemotherapy (Ribeiro et al., 2016; Ribeiro & Paredes, 2014; Sousa et al., 2020; Vieira et al., 2012).

Thus, first P-cad expression was assessed to prove that the silencing had been successfully achieved ($0,247 \pm 0,263$) (Figure 11).

Regarding epithelial markers, the absence of P-cad did not affect the expression of E-cad. However, in the case of EpCAM, we observed a decrease ($1 > 0,466 \pm 0,115$) on its expression when P-cad was silenced, and the same was found for OCLN ($1 > 0,614 \pm 0,166$) (Figure 11).

In the case of mesenchymal markers, we did not find any VIM expression in BT-20 cells. Regarding N-cad, we did not observe significant changes in its expression when P-cad was silenced (Figure 11).

However, regarding the transcription factor ZEB2, we saw that the absence of P-cad led to a significant decrease on its expression ($1 > 0,616 \pm 0,142$) (Figure 11).

Finally, concerning PSFs, we observed that P-cad silencing led to a significant decrease in Δ Np63 expression ($1 > 0,457 \pm 0,388$). However, for GRHL2 and Integrin $\beta 4$ the absence of P-cad did not significantly affect the expression of these markers. The same negative result was found for tAMPK; but in the case of pAMPK, we could observe that P-cad silencing led to a strong and significant increase on pAMPK levels ($1 < 3,714 \pm 0,969$) (Figure 11).

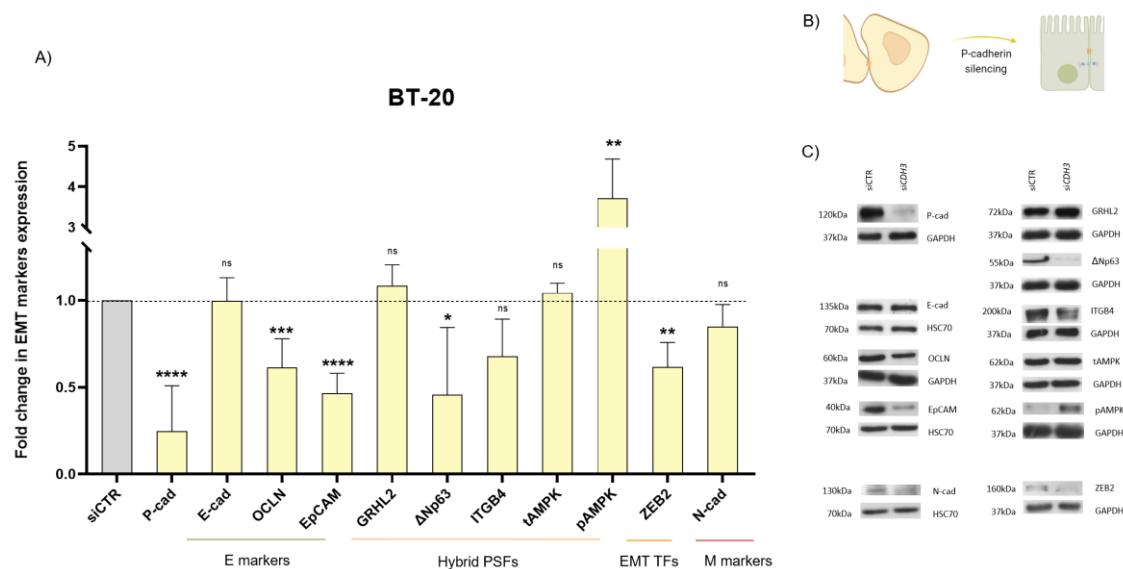


Figure 11. Evaluation of E and M markers, EMT TFs and hybrid PSFs expression in BT-20 cell line upon CDH3 silencing
A) Quantification of P-cadherin, E-cadherin, OCLN, EpCAM, GRHL2, Δ Np63, Integrin $\beta 4$, tAMPK, pAMPK, ZEB2 and N-cadherin proteins; (Mean $\pm$ SD, N = 6-6-5-5-3-4-3-4-3-3-4, n $\geq$ 3, Unpaired t test); (*p < 0.0332, **p < 0.0021, ***p < 0.0002, ****p < 0.0001). B) Schematic representation of cellular alterations when P-cad is silenced. [Image created in Biorender]. C) Representative images of Western Blot.

In this cell line, as expected, *CDH3* silencing induces a regression in EMT. The lower expression of P-cad, at an early stage of EMT, leads cells to acquire a more epithelial-like phenotype. Therefore, our results prove that P-cad plays a crucial role in the regulation of epithelial and mesenchymal markers, as well as TFs and PSFs (Figure 12).

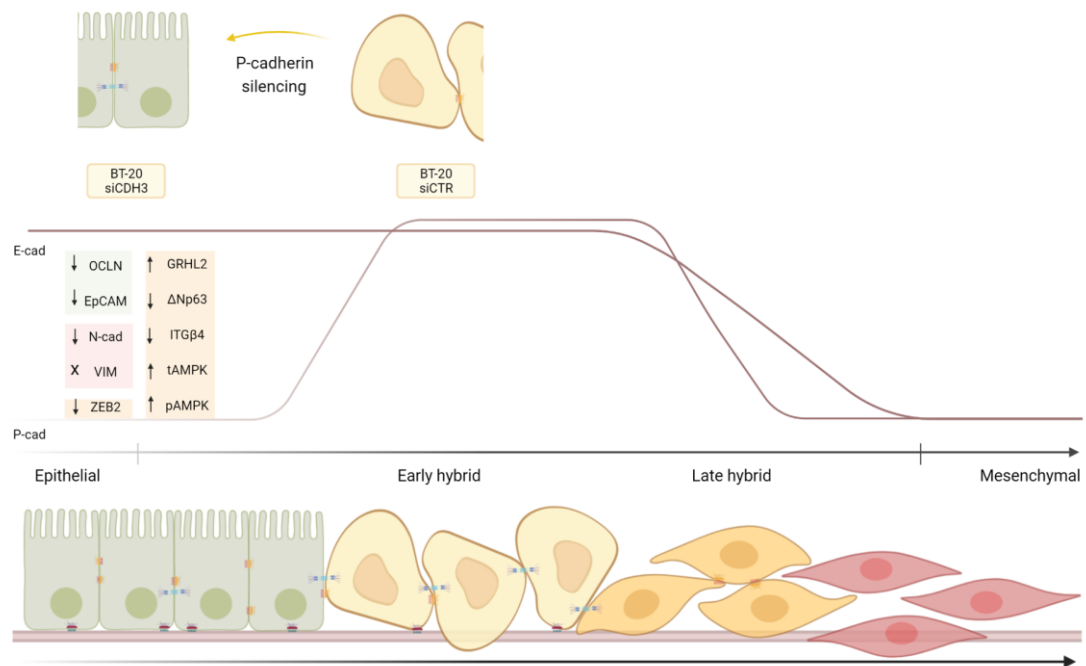


Figure 12. Schematic representation of the alteration in the expression of the different EMT markers with the silencing of P-cadherin in the BT-20 cell line. [Image created in Biorender].

4.2.3 SUM149 – a Basal B breast cancer cell line

The IBC SUM149 cell line represents a Basal B breast cancer subtype, although it is highly enriched for cell-cell adhesion molecules, such as E-cadherin and P-cadherin. Actually, it has been found that these cadherins are biomarkers of IBC emboli in circulation (Arora et al., 2017; Chakraborty, George, Woodward, Levine, & Jolly, 2021; Fernandez et al., 2013; Lehman et al., 2013).

Thus, P-cad expression was also silenced in this model to analyze its effect on EMT markers, since the mesenchymal ones are usually upregulated in Basal B cell lines. Again, we expected that P-cad silencing led cells to acquire a more epithelial-like phenotype.

The P-cad expression was analyzed to prove that the silencing had been successfully achieved ($0,483 \pm 0,212$) (Figure 13).

In the case of epithelial markers, the absence of P-cad did not affect the expression of E-cad and EpCAM, which are highly enriched in this cell line. However, regarding OCLN we observed a significant increase ($1 < 1,933 \pm 0,649$) on its expression, when P-cad was silenced (Figure 13).

Regarding mesenchymal markers, we observed the opposite findings, since the absence of P-cad led to a significant decrease in vimentin expression ($1 > 0,497 \pm 0,306$) but to a significant increase in N-cad expression ($1 < 1,662 \pm 0,518$) (Figure 13).

In the case of the transcription factor ZEB2, we saw that decreased levels of P-cad did not affect the expression of this marker (Figure 13).

Regarding the PSFs associated to partial EMT, we saw that P-cad silencing led to a significant increase in Δ Np63 expression ($1 < 1,621 \pm 0,525$). However, there were no changes in the expression of GRHL2 and tAMPK. For ITG β 4, we observed a significant decrease on its expression ($1 > 0,702 \pm 0,147$) due to P-cad absence, and the same occurred for pAMPK ($1 > 0,687 \pm 0,327$) (Figure 13).

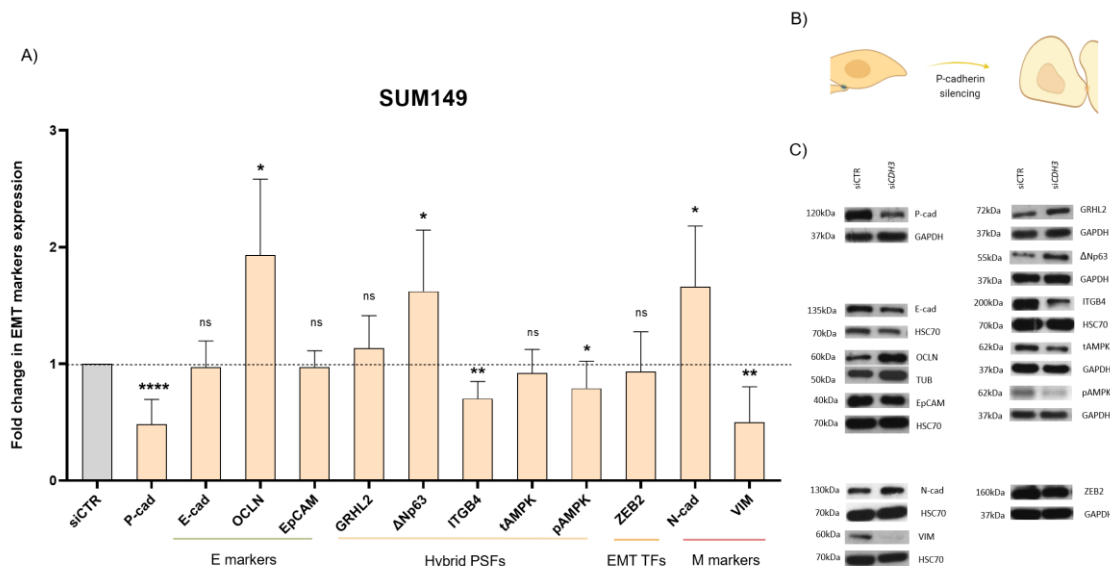


Figure 13. Evaluation of E and M markers, EMT TFs and hybrid PSFs expression in SUM149 cell line upon CDH3 silencing. A) Quantification of P-cadherin, E-cadherin, OCLN, EpCAM, GRHL2, GRHL2, Δ Np63, Integrin β 4, tAMPK, pAMPK, ZEB2, N-cadherin and Vimentin proteins; (Mean $\pm$ SD, N = 8-8-4-8-7-5-4-7-5-7-6-6, n $\geq$ 3, Unpaired t test); (* $p < 0.0332$, ** $p < 0.0021$, *** $p < 0.0002$, **** $p < 0.0001$). B) Schematic representation of cellular alterations when P-cad is silenced. [Image created in Biorender]. C) Representative images of Western Blot.

In the case of the SUM149 cell line, it was also expected that *CDH3* silencing would lead to a regression in EMT. And, in fact, our results show that the lower expression of P-cad leads cells to become more epithelial. However, SUM149 siCDH3 cells do not acquire an epithelial phenotype, but they seem to end up being stabilized in the early hy-phen (Figure 14).

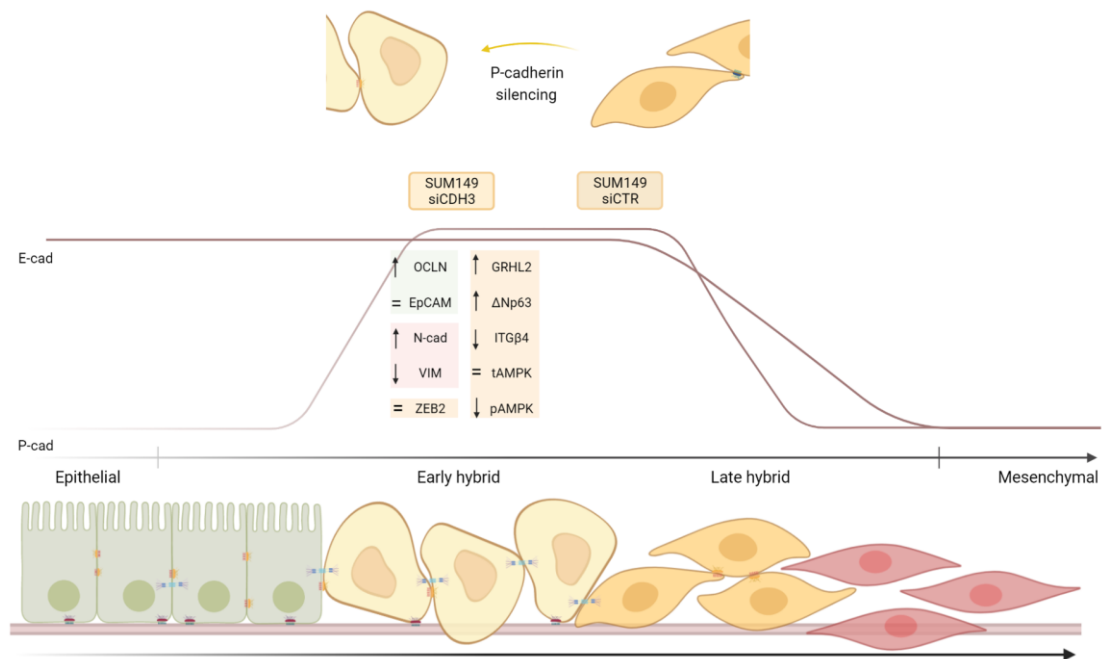


Figure 14. Schematic representation of the alteration in the expression of the different EMT markers with the silencing of P-cadherin in the SUM149 cell line. [Image created in Biorender].

5. General Discussion

P-cad expression is a poor prognostic factor for breast cancer patients and *in vitro* evidence has shown that P-cad-mediated cell-cell adhesion enables cancer cells to be enriched in stem-like properties, to be *anoikis*-resistant and to be invasive in a collective way. Although all these characteristics are enriched by cancer cells while these change their phenotype during EMT, little is known about P-cad's role during this process.

Thus, based on our previous data, the principal goal of this study was to investigate if P-cad expression could act as a PSF or even as a biomarker of a stable *hy-phen* in breast cancer cells, operating as a lever of phenotypic cell plasticity that drives metastatic aggressiveness. In order to achieve this main goal, two specific aims have been proposed: 1) to assess the correlation between P-cad expression and the expression of EMT markers in a panel of breast cancer cell lines; and 2) to investigate if P-cad expression would regulate EMT biomarkers, including PSFs associated to a stable hybrid E/M phenotype

To achieve the first aim, the CCLE online database has been used to study the correlation between mRNA expression of *CDH3* and the expression of several transcripts associated to EMT in a large panel of 57 breast cancer cell lines. As initially predicted, a positive association was found with more epithelial-like markers (*CDH1*, *OCLN*, *EPCAM*, *OVOL2*, *GRHL2*, *ITGB4* and *TP63*) and an inverse correlation was observed between *CDH3* expression and markers associated to mesenchymal cells (*CDH2*, *VCAM1*, *VIM*, *ITGB3*, *ZEB1* and *ZEB2*). Although not significant, there was also a trend for an inverse association with other EMT TFs, such as *TWIST1*, *SNAI1* and *SNAI2*, and for a direct association with *CTNNB1*. These first results support that P-cad is a marker enriched in cancer cells that still maintain cell-cell adhesion and an epithelial phenotype, being significantly associated to the early hybrid EMT markers *ITGB4*, *GRHL2*, *OVOL2* and *TP63*.

By applying the Neve classification to the CCLE series of breast cancer cell lines, we were also able to confirm that there is a different gene expression distribution for each biomarker dependent on the breast cancer subtype. As predicted, epithelial biomarkers were mainly enriched in Luminal and Basal A cell lines (*CDH1*, *OCLN* and *EPCAM*), whereas mesenchymal associated markers

were highly expressed in Basal B cell lines (*CDH2*, *VIM*, *CD44* and *ITGB3*, *ZEB2*, *ZEB1*, *SNAI1*, *SNAI2* and *TWIST1*). Concerning gene expression of putative biomarkers of an early hybrid EMT phenotype, we could find that *OVOL2*, *GRHL2* and *ITGB4* are highly expressed in Luminal and Basal A cell lines, whereas their expression is more dispersed within Basal B cell lines. Interestingly, *CDH3* was the only transcript that was actually highly enriched in Basal A cell lines in comparison to Luminal or Basal B cells, which support our hypothesis that *CDH3*/P-cadherin can be an important biomarker of cells harboring an EMT hybrid phenotype, since Basal A subtype is enriched in cell lines harboring epithelial and mesenchymal characteristics (Neve et al., 2006; Perou et al., 2000; Ricardo et al., 2011; Vieira et al., 2012; Voutilainen et al., 2021).

Interestingly, by studying the expression of some EMT markers in these 3 cell models, where *CDH3* expression was altered, we were able to draw common conclusions that support the initial hypothesis raised in this study.

Regarding E-cad expression we can see that its expression is always constant regardless the presence or absence of P-cad. As it is known, cells that co-express E- and P-cad are associated with a more aggressive phenotype and can migrate in clusters (Hapach et al., 2021; Padmanaban et al., 2019). Since, during EMT, cells finally lose E-cad expression to acquire a mesenchymal phenotype, we can assume that the co-expression of the two cadherins only occurs at an early stage of the EMT, because here the decrease in E-cad is still not seen. Interestingly, for EpCAM and OCLN epithelial markers, we did not find the same results. P-cad expression was actually able to increase their expression, which probably is important to allow cells to migrate collectively, supporting cell cluster migration (Boral et al., 2017; Curtaz et al., 2020). We can assume that P-cad plays an important role in inducing collective migration, by regulating the expression of some epithelial markers, such as EpCAM and OCLN.

Concerning N-cad, a mesenchymal marker, we observed that its expression is very weak in the two most epithelial-like cell lines and that there wasn't almost any changes on its expression upon *CDH3* overexpression or silencing. However, for SUM149 cells, when P-cad was silenced, N-cad expression was

increased. This may have occurred to counteract the loss of P-cad. So, in this case, N-cad may have a role as a cell-cell adhesion molecule in this context (Bruner & Derksen, 2018; Cao et al., 2019; Hazan, Qiao, Keren, Badano, & Suyama, 2004; Suyama, Shapiro, Guttman, & Hazan, 2002), supporting the cadherin switch mechanism already described between E-cad and N-cad.

Regarding the EMT transcription factor ZEB2, it is known that the increase on its expression indicates the suppression of epithelial markers, namely E-cad, which promotes EMT (He et al., 2017). Recent studies analyzed ZEB2 mRNA levels during EMT and found that when cells have a more epithelial-like phenotype they have lower levels of ZEB2, compared to hybrid E/M and mesenchymal phenotypes, where ZEB2 mRNA levels were respectively higher (Mooney et al., 2017). Therefore, our results are in line with what had been observed, since P-cad expression promotes the expression of this TF, leading EMT progression.

GRHL2, an early hybrid PSF plays a crucial role maintaining collective cell migration (Jolly et al., 2016; Varma et al., 2012). It is known that during EMT there is a mutual inhibition feedback loop between GRHL2 and ZEB1/2. Therefore, the lower expression of GRHL2 is essentially due to the increased expression of ZEB1/2 (Mooney et al., 2017). As we could observe in our results, regarding MCF-7/AZ cell line, P-cad overexpression led to a decrease in GRHL2 expression, which demonstrates an advance in EMT. In the case of the BT-20 and SUM149 cell lines, when P-cad was silenced, GRHL2 expression, although not significant, increased, which leads us to believe that there is a regression in the EMT. So, the decrease observed in GRHL2 expression when P-cad was overexpressed demonstrate an advance in EMT (Cieply et al., 2013; Mlacki et al., 2015).

In the case of Δ Np63, we observed the expression of isoform β in the two most epithelial-like cell lines and the isoform present in the SUM149 cell line is the α . However, both isoforms are recognized as EMT inhibitors (Lindsay et al., 2011) and, in early hy-phen, the presence of P-cad induces increased expression of this PSF. These results can indicate that Δ Np63, at an early stage of EMT, plays an important role in maintaining the hybrid phenotype. Therefore, cells in

this early hy-phen, end up being stabilized and not having a complete progression to the epithelial or to the mesenchymal phenotype. Although there is currently no proven relationship between this PSF and P-cad in cancer, other authors have already validated P-cadherin as a direct transcriptional target of p63 in other diseases (Shimomura, Wajid, Shapiro, & Christiano, 2008). Thus, based on these studies and in our results, we believe that perhaps there might be a feedback mechanism between these two proteins.

The decrease in pAMPK expression, when P-cad starts to be expressed can indicate that there is less phosphorylation of the raptor and less inhibition of mTOR (Gwinn et al., 2008; Jung, Ro, Cao, Otto, & Kim, 2010), which also induces lower autophagy compared to the epithelial phenotype or to the intermediate/late hy-phen. So, autophagy can play a dual role in cancer progression (Babaei, Aziz, & Jaghi, 2021; Colella, Faienza, & Di Bartolomeo, 2019; Marcucci, Ghezzi, & Rumio, 2017). At an earlier phase of EMT, we think that autophagy will have a role as tumor suppressor because, as observed in glioblastoma, the induction of autophagy, by inhibiting mTOR, leads to a reduction in cell migration and invasion so cells become less aggressive and apparently more epithelial (Catalano et al., 2015; Colella et al., 2019; Mecca et al., 2018). However, at a more advanced stage of the hybrid phenotype, autophagy may play a pro-oncogenic role, inducing cell survival (Colella et al., 2019; Garg, 2020; Zou et al., 2016). Due to this, depending on the EMT stage, this PSF has either a tumor suppressor or pro-oncogenic role, allowing cells to maintain a stable hy-phen.

In the case of VIM, since it is a mesenchymal marker, such as N-cad, its expression should be practically non-existent in the two most epithelial-like cell lines, however, this only occurs in BT-20 cells. Regarding MCF-7/AZ cell line, we verified a decrease in VIM expression when P-cad was overexpressed. For SUM149 cells, P-cad silencing also induces a decrease in VIM expression. It is known that lower expression of vimentin is associated with autophagosomes accumulation, which indicates an increase in autophagy (Biskou et al., 2019). It is also known that autophagy plays a dual role in cancer progression, however here, in both cell lines we believed that autophagy ends up having a tumor suppressor role (Garg, 2020; Klionsky et al., 2021).

Finally, for ITG β 4, we don't observe the same tendency when P-cad is silenced or overexpressed in the two most epithelial-like cell lines. In the case of MCF-7/AZ cells, we observed a decrease on its expression when P-cad was overexpressed, which ends up as having a putative inhibitory role in the progression of EMT. On the other hand, the decrease, although not significant, in ITG β 4 expression in BT-20 cells, can induce regression and inhibition of EMT. Since ITG β 4 is a PSF, its goal is to stabilize the hybrid phenotype. Therefore, there are cases where this marker is eventually used as a marker of epithelial and hybrid phenotype (Kröger et al., 2019) but, and in other cases its upregulation promotes EMT (Masugi et al., 2015) and tumor growth (X. L. Li et al., 2017). Regarding SUM149 cell line, the decrease in ITG β 4 expression upon P-cad silencing induce regression and inhibition of EMT (X. L. Li et al., 2017; Masugi et al., 2015), which indicates that cells become more epithelial when P-cad is silenced.

In general, we observed that in the different breast cancer cell lines the expression of P-cad promotes the expression of epithelial markers and a decrease in the mesenchymal ones. However, since we know that its expression also induces collective cell invasion, it seems that actually P-cad can be associated to the maintenance of an early hybrid EMT phenotype. It was very interesting to find that its expression was significantly associated to the expression of the EMT TF ZEB2, as well as to the early hybrid EMT factor Δ Np63 (Figure 15). Moreover, P-cad overexpression seems also be able to regulate pAMPK and GRHL2 by inducing a lower expression of both (Figure 15). In the future, further mechanistic studies are needed to better explore these results and understand how and where P-cad expression acts during the EMT process.

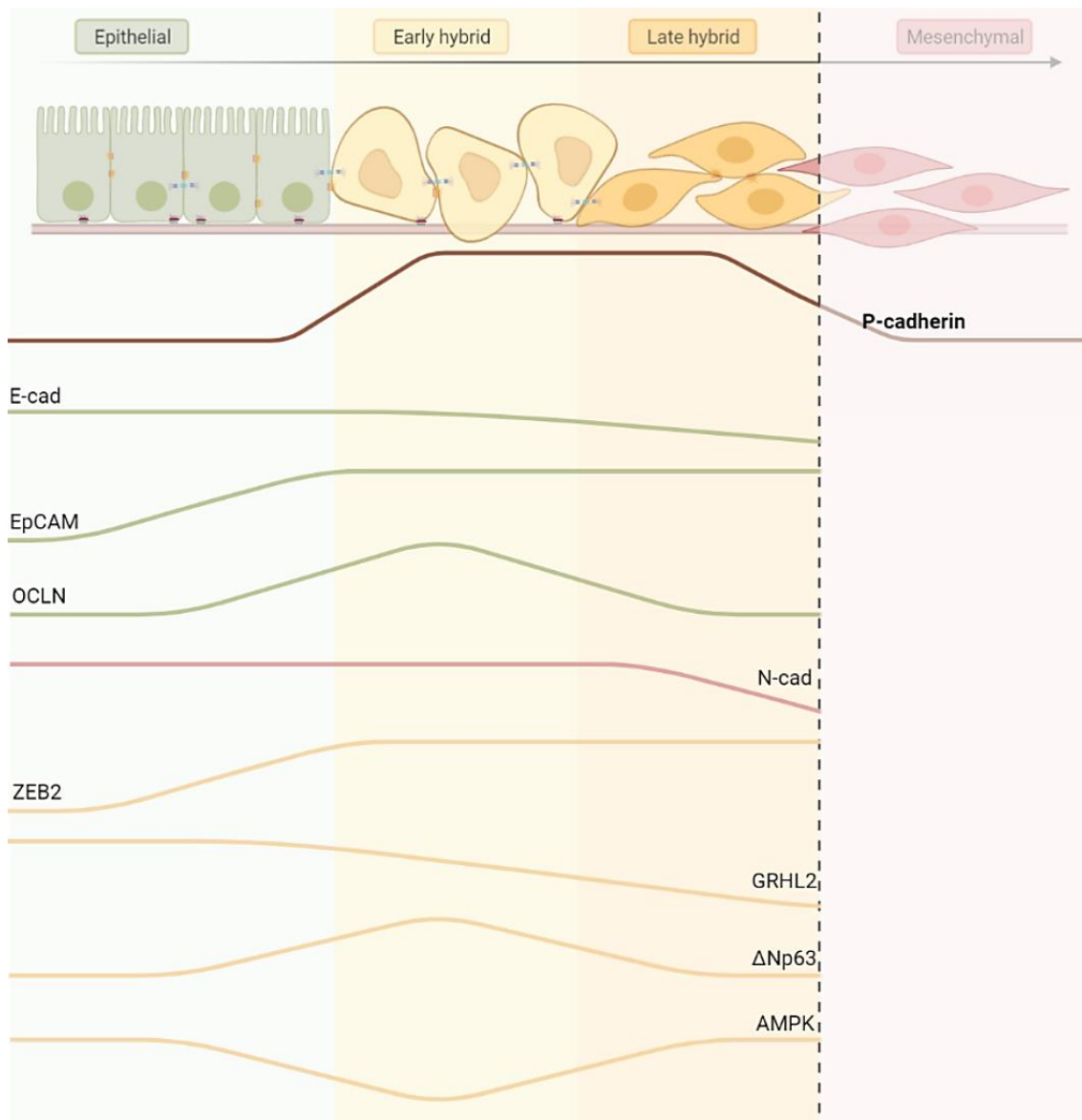


Figure 15. Schematic representation of changes in the expression of the different EMT markers. Changes in the expression of EMT markers, depending on the change in P-cad expression, in the three breast cancer cell lines. [Image created in Biorender].

6. References

- Aban, C. E., Lombardi, A., Neiman, G., Biani, M. C., La Greca, A., Waisman, A., . . . Luzzani, C. (2021). Downregulation of E-cadherin in pluripotent stem cells triggers partial EMT. *Sci Rep*, 11(1), 2048. doi:10.1038/s41598-021-81735-1
- Aceto, N., Bardia, A., Miyamoto, D. T., Donaldson, M. C., Wittner, B. S., Spencer, J. A., . . . Maheswaran, S. (2014). Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. *Cell*, 158(5), 1110-1122. doi:10.1016/j.cell.2014.07.013
- Acloque, H., Ocaña, O. H., Abad, D., Stern, C. D., & Nieto, M. A. (2017). Snail2 and Zeb2 repress P-cadherin to define embryonic territories in the chick embryo. *Development*, 144(4), 649-656. doi:10.1242/dev.142562
- Ades, F., Zardavas, D., Bozovic-Spasojevic, I., Pugliano, L., Fumagalli, D., de Azambuja, E., . . . Piccart, M. (2014). Luminal B breast cancer: molecular characterization, clinical management, and future perspectives. *J Clin Oncol*, 32(25), 2794-2803. doi:10.1200/jco.2013.54.1870
- Agarwal, A., Balic, M., El-Ashry, D., & Cote, R. J. (2018). Circulating Tumor Cells: Strategies for Capture, Analyses, and Propagation. *Cancer J*, 24(2), 70-77. doi:10.1097/ppo.0000000000000310
- Al-Hajj, M., Wicha, M. S., Benito-Hernandez, A., Morrison, S. J., & Clarke, M. F. (2003). Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci U S A*, 100(7), 3983-3988. doi:10.1073/pnas.0530291100
- Albergaria, A., Ribeiro, A. S., Vieira, A. F., Sousa, B., Nobre, A. R., Seruca, R., . . . Paredes, J. (2011). P-cadherin role in normal breast development and cancer. *Int J Dev Biol*, 55(7-9), 811-822. doi:10.1387/ijdb.113382aa
- Alves, C. C., Carneiro, F., Hoefler, H., & Becker, K. F. (2009). Role of the epithelial-mesenchymal transition regulator Slug in primary human cancers. *Front Biosci (Landmark Ed)*, 14, 3035-3050. doi:10.2741/3433
- Angers, S., & Moon, R. T. (2009). Proximal events in Wnt signal transduction. *Nat Rev Mol Cell Biol*, 10(7), 468-477. doi:10.1038/nrm2717
- Arenas, M. I., Romo, E., Royuela, M., Fraile, B., & Paniagua, R. (2000). E-, N- and P-cadherin, and alpha-, beta- and gamma-catenin protein expression in normal, hyperplastic and carcinomatous human prostate. *Histochem J*, 32(11), 659-667. doi:10.1023/a:1004111331752
- Arora, J., Sauer, S. J., Tarpley, M., Vermeulen, P., Rypens, C., Van Laere, S., . . . Dewhirst, M. W. (2017). Inflammatory breast cancer tumor emboli express high levels of anti-apoptotic proteins: use of a quantitative high content and high-throughput 3D IBC spheroid assay to identify targeting strategies. *Oncotarget*, 8(16), 25848-25863. doi:10.18632/oncotarget.15667
- Assani, G., & Zhou, Y. (2019). Effect of modulation of epithelial-mesenchymal transition regulators Snail1 and Snail2 on cancer cell radiosensitivity by targeting of the cell cycle, cell apoptosis and cell migration/invasion. *Oncol Lett*, 17(1), 23-30. doi:10.3892/ol.2018.9636
- Ataollahi, M. R., Sharifi, J., Paknahad, M. R., & Paknahad, A. (2015). Breast cancer and associated factors: a review. *J Med Life*, 8(Spec Iss 4), 6-11.
- Babaei, G., Aziz, S. G., & Jaghi, N. Z. Z. (2021). EMT, cancer stem cells and autophagy; The three main axes of metastasis. *Biomed Pharmacother*, 133, 110909. doi:10.1016/j.biopha.2020.110909
- Bacac, M., & Stamenkovic, I. (2008). Metastatic cancer cell. *Annu Rev Pathol*, 3, 221-247. doi:10.1146/annurev.pathmechdis.3.121806.151523
- Bai, R., Kusama, K., Nakamura, K., Sakurai, T., Kimura, K., Ideta, A., . . . Imakawa, K. (2018). Down-regulation of transcription factor OVOL2 contributes to epithelial-mesenchymal

- transition in a noninvasive type of trophoblast implantation to the maternal endometrium. *Faseb j*, 32(6), 3371-3384. doi:10.1096/fj.201701131RR
- Bakir, B., Chiarella, A. M., Pitarresi, J. R., & Rustgi, A. K. (2020). EMT, MET, Plasticity, and Tumor Metastasis. *Trends Cell Biol*, 30(10), 764-776. doi:10.1016/j.tcb.2020.07.003
- Banys-Paluchowski, M., Krawczyk, N., Meier-Stiegen, F., & Fehm, T. (2016). Circulating tumor cells in breast cancer--current status and perspectives. *Crit Rev Oncol Hematol*, 97, 22-29. doi:10.1016/j.critrevonc.2015.10.010
- Baumeister, P., Hollmann, A., Kitz, J., Afthonidou, A., Simon, F., Shakhtour, J., . . . Gires, O. (2018). High Expression of EpCAM and Sox2 is a Positive Prognosticator of Clinical Outcome for Head and Neck Carcinoma. *Sci Rep*, 8(1), 14582. doi:10.1038/s41598-018-32178-8
- Behrens, J. (1999). Cadherins and catenins: role in signal transduction and tumor progression. *Cancer Metastasis Rev*, 18(1), 15-30. doi:10.1023/a:1006200102166
- Bidard, F. C., Peeters, D. J., Fehm, T., Nol  , F., Gisbert-Criado, R., Mavroudis, D., . . . Michiels, S. (2014). Clinical validity of circulating tumour cells in patients with metastatic breast cancer: a pooled analysis of individual patient data. *Lancet Oncol*, 15(4), 406-414. doi:10.1016/s1470-2045(14)70069-5
- Biskou, O., Casanova, V., Hooper, K. M., Kemp, S., Wright, G. P., Satsangi, J., . . . Stevens, C. (2019). The type III intermediate filament vimentin regulates organelle distribution and modulates autophagy. *PLoS One*, 14(1), e0209665. doi:10.1371/journal.pone.0209665
- Biswas, K., Jolly, M. K., & Ghosh, A. (2019). Stability and mean residence times for hybrid epithelial/mesenchymal phenotype. *Phys Biol*, 16(2), 025003. doi:10.1088/1478-3975/aaf7b7
- Blanco, M. J., Moreno-Bueno, G., Sarrio, D., Locascio, A., Cano, A., Palacios, J., & Nieto, M. A. (2002). Correlation of Snail expression with histological grade and lymph node status in breast carcinomas. *Oncogene*, 21(20), 3241-3246. doi:10.1038/sj.onc.1205416
- Bocci, F., Jolly, M. K., Tripathi, S. C., Aguilar, M., Hanash, S. M., Levine, H., & Onuchic, J. N. (2017). Numb prevents a complete epithelial-mesenchymal transition by modulating Notch signalling. *J R Soc Interface*, 14(136). doi:10.1098/rsif.2017.0512
- Bocci, F., Tripathi, S. C., Vilchez Mercedes, S. A., George, J. T., Casabar, J. P., Wong, P. K., . . . Jolly, M. K. (2019). NRF2 activates a partial epithelial-mesenchymal transition and is maximally present in a hybrid epithelial/mesenchymal phenotype. *Integr Biol (Camb)*, 11(6), 251-263. doi:10.1093/intbio/zyz021
- Bol  s, V., Peinado, H., P  rez-Moreno, M. A., Fraga, M. F., Esteller, M., & Cano, A. (2003). The transcription factor Slug represses E-cadherin expression and induces epithelial to mesenchymal transitions: a comparison with Snail and E47 repressors. *J Cell Sci*, 116(Pt 3), 499-511. doi:10.1242/jcs.00224
- Boral, D., Vishnoi, M., Liu, H. N., Yin, W., Sprouse, M. L., Scamardo, A., . . . Marchetti, D. (2017). Molecular characterization of breast cancer CTCs associated with brain metastasis. *Nat Commun*, 8(1), 196. doi:10.1038/s41467-017-00196-1
- Bottoni, P., Isgr  , M. A., & Scatena, R. (2016). The epithelial-mesenchymal transition in cancer: a potential critical topic for translational proteomic research. *Expert Rev Proteomics*, 13(1), 115-133. doi:10.1586/14789450.2016.1112742
- Brabletz, S., Schuhwerk, H., Brabletz, T., & Stemmler, M. P. (2021). Dynamic EMT: a multi-tool for tumor progression. *Embo j*, e108647. doi:10.15252/embj.2021108647
- Brown, R. L., Reinke, L. M., Damerow, M. S., Perez, D., Chodosh, L. A., Yang, J., & Cheng, C. (2011). CD44 splice isoform switching in human and mouse epithelium is essential for epithelial-mesenchymal transition and breast cancer progression. *J Clin Invest*, 121(3), 1064-1074. doi:10.1172/jci44540
- Browne, G., Sayan, A. E., & Tulchinsky, E. (2010). ZEB proteins link cell motility with cell cycle control and cell survival in cancer. *Cell Cycle*, 9(5), 886-891. doi:10.4161/cc.9.5.10839

- Bruner, H. C., & Derksen, P. W. B. (2018). Loss of E-Cadherin-Dependent Cell-Cell Adhesion and the Development and Progression of Cancer. *Cold Spring Harb Perspect Biol*, 10(3). doi:10.1101/cshperspect.a029330
- Byrne, G. J., Ghellal, A., Iddon, J., Blann, A. D., Venizelos, V., Kumar, S., . . . Bundred, N. J. (2000). Serum soluble vascular cell adhesion molecule-1: role as a surrogate marker of angiogenesis. *J Natl Cancer Inst*, 92(16), 1329-1336. doi:10.1093/jnci/92.16.1329
- Cabel, L., Proudhon, C., Gortais, H., Loirat, D., Coussy, F., Pierga, J. Y., & Bidard, F. C. (2017). Circulating tumor cells: clinical validity and utility. *Int J Clin Oncol*, 22(3), 421-430. doi:10.1007/s10147-017-1105-2
- Cao, Z. Q., Wang, Z., & Leng, P. (2019). Aberrant N-cadherin expression in cancer. *Biomed Pharmacother*, 118, 109320. doi:10.1016/j.biopha.2019.109320
- Carico, E., French, D., Bucci, B., Falcioni, R., Vecchione, A., & Mariani-Costantini, R. (1993). Integrin beta 4 expression in the neoplastic progression of cervical epithelium. *Gynecol Oncol*, 49(1), 61-66. doi:10.1006/gyno.1993.1087
- Casas, E., Kim, J., Bendesky, A., Ohno-Machado, L., Wolfe, C. J., & Yang, J. (2011). Snail2 is an essential mediator of Twist1-induced epithelial mesenchymal transition and metastasis. *Cancer Res*, 71(1), 245-254. doi:10.1158/0008-5472.Can-10-2330
- Catalano, M., D'Alessandro, G., Lepore, F., Corazzari, M., Caldarola, S., Valacca, C., . . . Di Bartolomeo, S. (2015). Autophagy induction impairs migration and invasion by reversing EMT in glioblastoma cells. *Mol Oncol*, 9(8), 1612-1625. doi:10.1016/j.molonc.2015.04.016
- Chakrabarti, R., Wei, Y., Hwang, J., Hang, X., Andres Blanco, M., Choudhury, A., . . . Kang, Y. (2014). ΔNp63 promotes stem cell activity in mammary gland development and basal-like breast cancer by enhancing Fzd7 expression and Wnt signalling. *Nat Cell Biol*, 16(10), 1004-1015, 1001-1013. doi:10.1038/ncb3040
- Chakraborty, P., George, J. T., Woodward, W. A., Levine, H., & Jolly, M. K. (2021). Gene expression profiles of inflammatory breast cancer reveal high heterogeneity across the epithelial-hybrid-mesenchymal spectrum. *Transl Oncol*, 14(4), 101026. doi:10.1016/j.tranon.2021.101026
- Chambers, A. F., Groom, A. C., & MacDonald, I. C. (2002). Dissemination and growth of cancer cells in metastatic sites. *Nat Rev Cancer*, 2(8), 563-572. doi:10.1038/nrc865
- Che, N., Zhao, X. L., Sun, T., Zhao, X. M., Gu, Q., Dong, X. Y., . . . Sun, B. C. (2011). The role of Twist1 in hepatocellular carcinoma angiogenesis: a clinical study. *Hum Pathol*, 42(6), 840-847. doi:10.1016/j.humpath.2010.03.010
- Chen, M. B., Wu, X. Y., Gu, J. H., Guo, Q. T., Shen, W. X., & Lu, P. H. (2011). Activation of AMP-activated protein kinase contributes to doxorubicin-induced cell death and apoptosis in cultured myocardial H9c2 cells. *Cell Biochem Biophys*, 60(3), 311-322. doi:10.1007/s12013-011-9153-0
- Chen, Q., Manning, C. D., Millar, H., McCabe, F. L., Ferrante, C., Sharp, C., . . . Anderson, G. M. (2008). CNTO 95, a fully human anti alphav integrin antibody, inhibits cell signaling, migration, invasion, and spontaneous metastasis of human breast cancer cells. *Clin Exp Metastasis*, 25(2), 139-148. doi:10.1007/s10585-007-9132-4
- Chen, Q., & Massagué, J. (2012). Molecular pathways: VCAM-1 as a potential therapeutic target in metastasis. *Clin Cancer Res*, 18(20), 5520-5525. doi:10.1158/1078-0432.Ccr-11-2904
- Chen, Q., Zhang, X. H., & Massagué, J. (2011). Macrophage binding to receptor VCAM-1 transmits survival signals in breast cancer cells that invade the lungs. *Cancer Cell*, 20(4), 538-549. doi:10.1016/j.ccr.2011.08.025
- Chen, T., You, Y., Jiang, H., & Wang, Z. Z. (2017). Epithelial-mesenchymal transition (EMT): A biological process in the development, stem cell differentiation, and tumorigenesis. *J Cell Physiol*, 232(12), 3261-3272. doi:10.1002/jcp.25797

- Chen, W., Yi, J. K., Shimane, T., Mehrazarin, S., Lin, Y. L., Shin, K. H., . . . Kang, M. K. (2016). Grainyhead-like 2 regulates epithelial plasticity and stemness in oral cancer cells. *Carcinogenesis*, 37(5), 500-510. doi:10.1093/carcin/bgw027
- Chen, Z., Fang, Z., & Ma, J. (2021). Regulatory mechanisms and clinical significance of vimentin in breast cancer. *Biomed Pharmacother*, 133, 111068. doi:10.1016/j.biopha.2020.111068
- Chen, Z. F., & Behringer, R. R. (1995). twist is required in head mesenchyme for cranial neural tube morphogenesis. *Genes Dev*, 9(6), 686-699. doi:10.1101/gad.9.6.686
- Cheng, G. Z., Chan, J., Wang, Q., Zhang, W., Sun, C. D., & Wang, L. H. (2007). Twist transcriptionally up-regulates AKT2 in breast cancer cells leading to increased migration, invasion, and resistance to paclitaxel. *Cancer Res*, 67(5), 1979-1987. doi:10.1158/0008-5472.Can-06-1479
- Cheuk, I. W., Siu, M. T., Ho, J. C., Chen, J., Shin, V. Y., & Kwong, A. (2020). ITGAV targeting as a therapeutic approach for treatment of metastatic breast cancer. *Am J Cancer Res*, 10(1), 211-223.
- Cheung, K. J., & Ewald, A. J. (2016). A collective route to metastasis: Seeding by tumor cell clusters. *Science*, 352(6282), 167-169. doi:10.1126/science.aaf6546
- Cheung, K. J., Gabrielson, E., Werb, Z., & Ewald, A. J. (2013). Collective invasion in breast cancer requires a conserved basal epithelial program. *Cell*, 155(7), 1639-1651. doi:10.1016/j.cell.2013.11.029
- Cheung, L. W., Leung, P. C., & Wong, A. S. (2010). Cadherin switching and activation of p120 catenin signaling are mediators of gonadotropin-releasing hormone to promote tumor cell migration and invasion in ovarian cancer. *Oncogene*, 29(16), 2427-2440. doi:10.1038/onc.2009.523
- Cheung, M., Chaboissier, M. C., Mynett, A., Hirst, E., Schedl, A., & Briscoe, J. (2005). The transcriptional control of trunk neural crest induction, survival, and delamination. *Dev Cell*, 8(2), 179-192. doi:10.1016/j.devcel.2004.12.010
- Cieply, B., Farris, J., Denvir, J., Ford, H. L., & Frisch, S. M. (2013). Epithelial-mesenchymal transition and tumor suppression are controlled by a reciprocal feedback loop between ZEB1 and Grainyhead-like-2. *Cancer Res*, 73(20), 6299-6309. doi:10.1158/0008-5472.Can-12-4082
- Cieply, B., Riley, P. t., Pifer, P. M., Widmeyer, J., Addison, J. B., Ivanov, A. V., . . . Frisch, S. M. (2012). Suppression of the epithelial-mesenchymal transition by Grainyhead-like-2. *Cancer Res*, 72(9), 2440-2453. doi:10.1158/0008-5472.Can-11-4038
- Colella, B., Faienza, F., & Di Bartolomeo, S. (2019). EMT Regulation by Autophagy: A New Perspective in Glioblastoma Biology. *Cancers (Basel)*, 11(3). doi:10.3390/cancers11030312
- Comijn, J., Berx, G., Vermassen, P., Verschueren, K., van Grunsven, L., Bruyneel, E., . . . van Roy, F. (2001). The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion. *Mol Cell*, 7(6), 1267-1278. doi:10.1016/s1097-2765(01)00260-x
- Coopman, P., & Djiane, A. (2016). Adherens Junction and E-Cadherin complex regulation by epithelial polarity. *Cell Mol Life Sci*, 73(18), 3535-3553. doi:10.1007/s00018-016-2260-8
- Creighton, C. J., Fu, X., Hennessy, B. T., Casa, A. J., Zhang, Y., Gonzalez-Angulo, A. M., . . . Schiff, R. (2010). Proteomic and transcriptomic profiling reveals a link between the PI3K pathway and lower estrogen-receptor (ER) levels and activity in ER+ breast cancer. *Breast Cancer Res*, 12(3), R40. doi:10.1186/bcr2594
- Curtaz, C. J., Schmitt, C., Herbert, S. L., Feldheim, J., Schlegel, N., Gosselet, F., . . . Burek, M. (2020). Serum-derived factors of breast cancer patients with brain metastases alter permeability of a human blood-brain barrier model. *Fluids Barriers CNS*, 17(1), 31. doi:10.1186/s12987-020-00192-6

- Curtis, C., Shah, S. P., Chin, S. F., Turashvili, G., Rueda, O. M., Dunning, M. J., . . . Aparicio, S. (2012). The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature*, 486(7403), 346-352. doi:10.1038/nature10983
- Cyprian, F. S., Akhtar, S., Gatalica, Z., & Vranic, S. (2019). Targeted immunotherapy with a checkpoint inhibitor in combination with chemotherapy: A new clinical paradigm in the treatment of triple-negative breast cancer. *Bosn J Basic Med Sci*, 19(3), 227-233. doi:10.17305/bjbms.2019.4204
- Dai, X., Xin, Y., Xu, W., Tian, X., Wei, X., & Zhang, H. (2021). CBP-mediated Slug acetylation stabilizes Slug and promotes EMT and migration of breast cancer cells. *Sci China Life Sci*, 64(4), 563-574. doi:10.1007/s11427-020-1736-5
- Dang, T. T., Esparza, M. A., Maine, E. A., Westcott, J. M., & Pearson, G. W. (2015). Δ Np63 α Promotes Breast Cancer Cell Motility through the Selective Activation of Components of the Epithelial-to-Mesenchymal Transition Program. *Cancer Res*, 75(18), 3925-3935. doi:10.1158/0008-5472.Can-14-3363
- Deep, G., Jain, A. K., Ramteke, A., Ting, H., Vijendra, K. C., Gangar, S. C., . . . Agarwal, R. (2014). SNAI1 is critical for the aggressiveness of prostate cancer cells with low E-cadherin. *Mol Cancer*, 13, 37. doi:10.1186/1476-4598-13-37
- Dent, R., Trudeau, M., Pritchard, K. I., Hanna, W. M., Kahn, H. K., Sawka, C. A., . . . Narod, S. A. (2007). Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res*, 13(15 Pt 1), 4429-4434. doi:10.1158/1078-0432.Ccr-06-3045
- Di Giacomo, V., Tian, T. V., Mas, A., Pecoraro, M., Batlle-Morera, L., Noya, L., . . . Keyes, W. M. (2017). Δ Np63 α promotes adhesion of metastatic prostate cancer cells to the bone through regulation of CD82. *Oncogene*, 36(31), 4381-4392. doi:10.1038/onc.2017.42
- Dohn, M., Zhang, S., & Chen, X. (2001). p63 α and Δ Np63 α can induce cell cycle arrest and apoptosis and differentially regulate p53 target genes. *Oncogene*, 20(25), 3193-3205. doi:10.1038/sj.onc.1204427
- Dongre, A., & Weinberg, R. A. (2019). New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. *Nat Rev Mol Cell Biol*, 20(2), 69-84. doi:10.1038/s41580-018-0080-4
- Drivalos, A., Chrisofos, M., Efstathiou, E., Kapranou, A., Kollaitis, G., Koutlis, G., . . . Bamias, A. (2016). Expression of α 5-integrin, α 7-integrin, E-cadherin, and N-cadherin in localized prostate cancer. *Urol Oncol*, 34(4), 165.e111-168. doi:10.1016/j.urolonc.2015.10.016
- Duffy, M. J., McGowan, P. M., & Gallagher, W. M. (2008). Cancer invasion and metastasis: changing views. *J Pathol*, 214(3), 283-293. doi:10.1002/path.2282
- Eble, J. A., & Niland, S. (2019). The extracellular matrix in tumor progression and metastasis. *Clin Exp Metastasis*, 36(3), 171-198. doi:10.1007/s10585-019-09966-1
- Elisha, Y., Kalchenko, V., Kuznetsov, Y., & Geiger, B. (2018). Dual role of E-cadherin in the regulation of invasive collective migration of mammary carcinoma cells. *Sci Rep*, 8(1), 4986. doi:10.1038/s41598-018-22940-3
- Elloul, S., Elstrand, M. B., Nesland, J. M., Tropé, C. G., Kvalheim, G., Goldberg, I., . . . Davidson, B. (2005). Snail, Slug, and Smad-interacting protein 1 as novel parameters of disease aggressiveness in metastatic ovarian and breast carcinoma. *Cancer*, 103(8), 1631-1643. doi:10.1002/cncr.20946
- Eramo, A., Lotti, F., Sette, G., Piloizzi, E., Biffoni, M., Di Virgilio, A., . . . De Maria, R. (2008). Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell Death Differ*, 15(3), 504-514. doi:10.1038/sj.cdd.4402283
- Escrivá-de-Romaní, S., Arumí, M., Bellet, M., & Saura, C. (2018). HER2-positive breast cancer: Current and new therapeutic strategies. *Breast*, 39, 80-88. doi:10.1016/j.breast.2018.03.006
- Fabisiewicz, A., & Grzybowska, E. (2017). CTC clusters in cancer progression and metastasis. *Med Oncol*, 34(1), 12. doi:10.1007/s12032-016-0875-0

- Faddaoui, A., Sheta, R., Bachvarova, M., Plante, M., Gregoire, J., Renaud, M. C., . . . Bachvarov, D. (2017). Suppression of the grainyhead transcription factor 2 gene (GRHL2) inhibits the proliferation, migration, invasion and mediates cell cycle arrest of ovarian cancer cells. *Cell Cycle*, 16(7), 693-706. doi:10.1080/15384101.2017.1295181
- Feldman, G. J., Mullin, J. M., & Ryan, M. P. (2005). Occludin: structure, function and regulation. *Adv Drug Deliv Rev*, 57(6), 883-917. doi:10.1016/j.addr.2005.01.009
- Fernandez, S. V., Robertson, F. M., Pei, J., Aburto-Chumpitaz, L., Mu, Z., Chu, K., . . . Cristofanilli, M. (2013). Inflammatory breast cancer (IBC): clues for targeted therapies. *Breast Cancer Res Treat*, 140(1), 23-33. doi:10.1007/s10549-013-2600-4
- Fragomeni, S. M., Sciallis, A., & Jeruss, J. S. (2018). Molecular Subtypes and Local-Regional Control of Breast Cancer. *Surg Oncol Clin N Am*, 27(1), 95-120. doi:10.1016/j.soc.2017.08.005
- Fu, H., Qi, L., Chen, L., He, Y., Zhang, N., & Guo, H. (2016). Expression of Ovof2 is related to epithelial characteristics and shows a favorable clinical outcome in hepatocellular carcinoma. *Onco Targets Ther*, 9, 5963-5973. doi:10.2147/ott.S110409
- Fu, J., Qin, L., He, T., Qin, J., Hong, J., Wong, J., . . . Xu, J. (2011). The TWIST/Mi2/NuRD protein complex and its essential role in cancer metastasis. *Cell Res*, 21(2), 275-289. doi:10.1038/cr.2010.118
- Fukushi, J., Ono, M., Morikawa, W., Iwamoto, Y., & Kuwano, M. (2000). The activity of soluble VCAM-1 in angiogenesis stimulated by IL-4 and IL-13. *J Immunol*, 165(5), 2818-2823. doi:10.4049/jimmunol.165.5.2818
- Galimberti, V., Vicini, E., Corso, G., Morigi, C., Fontana, S., Sacchini, V., & Veronesi, P. (2017). Nipple-sparing and skin-sparing mastectomy: Review of aims, oncological safety and contraindications. *Breast*, 34 Suppl 1(Suppl 1), S82-s84. doi:10.1016/j.breast.2017.06.034
- Gallagher, A. J., & Schiemann, W. P. (2006). Beta3 integrin and Src facilitate transforming growth factor-beta mediated induction of epithelial-mesenchymal transition in mammary epithelial cells. *Breast Cancer Res*, 8(4), R42. doi:10.1186/bcr1524
- Gamallo, C., Moreno-Bueno, G., Sarrió, D., Calero, F., Hardisson, D., & Palacios, J. (2001). The prognostic significance of P-cadherin in infiltrating ductal breast carcinoma. *Mod Pathol*, 14(7), 650-654. doi:10.1038/modpathol.3880367
- Gao, J. J., & Swain, S. M. (2018). Luminal A Breast Cancer and Molecular Assays: A Review. *Oncologist*, 23(5), 556-565. doi:10.1634/theoncologist.2017-0535
- García, S. A., Weitz, J., & Schölch, S. (2018). Circulating Tumor Cells. *Methods Mol Biol*, 1692, 213-219. doi:10.1007/978-1-4939-7401-6_18
- Garg, M. (2017). Epithelial, mesenchymal and hybrid epithelial/mesenchymal phenotypes and their clinical relevance in cancer metastasis. *Expert Rev Mol Med*, 19, e3. doi:10.1017/erm.2017.6
- Garg, M. (2020). Epithelial Plasticity, Autophagy and Metastasis: Potential Modifiers of the Crosstalk to Overcome Therapeutic Resistance. *Stem Cell Rev Rep*, 16(3), 503-510. doi:10.1007/s12015-019-09945-9
- Giacobbe, A., Compagnone, M., Bongiorno-Borbone, L., Antonov, A., Markert, E. K., Zhou, J. H., . . . Peschiaroli, A. (2016). p63 controls cell migration and invasion by transcriptional regulation of MTSS1. *Oncogene*, 35(12), 1602-1608. doi:10.1038/onc.2015.230
- Gilles, C., Polette, M., Mestdagt, M., Nawrocki-Raby, B., Ruggeri, P., Birembaut, P., & Foidart, J. M. (2003). Transactivation of vimentin by beta-catenin in human breast cancer cells. *Cancer Res*, 63(10), 2658-2664.
- Gires, O., Pan, M., Schinke, H., Canis, M., & Baeuerle, P. A. (2020). Expression and function of epithelial cell adhesion molecule EpCAM: where are we after 40 years? *Cancer Metastasis Rev*, 39(3), 969-987. doi:10.1007/s10555-020-09898-3

- Gires, O., & Stoecklein, N. H. (2014). Dynamic EpCAM expression on circulating and disseminating tumor cells: causes and consequences. *Cell Mol Life Sci*, 71(22), 4393-4402. doi:10.1007/s00018-014-1693-1
- Gonzalez, D. M., & Medici, D. (2014). Signaling mechanisms of the epithelial-mesenchymal transition. *Sci Signal*, 7(344), re8. doi:10.1126/scisignal.2005189
- Goossens, S., Vandamme, N., Van Vlierberghe, P., & Berx, G. (2017). EMT transcription factors in cancer development re-evaluated: Beyond EMT and MET. *Biochim Biophys Acta Rev Cancer*, 1868(2), 584-591. doi:10.1016/j.bbcan.2017.06.006
- Gorges, T. M., Tinhofer, I., Drosch, M., Röse, L., Zollner, T. M., Krahn, T., & von Ahsen, O. (2012). Circulating tumour cells escape from EpCAM-based detection due to epithelial-to-mesenchymal transition. *BMC Cancer*, 12, 178. doi:10.1186/1471-2407-12-178
- Gu, A., Jie, Y., Yao, Q., Zhang, Y., & Mingyan, E. (2017). Slug Is Associated With Tumor Metastasis and Angiogenesis in Ovarian Cancer. *Reprod Sci*, 24(2), 291-299. doi:10.1177/1933719116654989
- Gu, J., Groot, G., Boden, C., Busch, A., Holtslander, L., & Lim, H. (2018). Review of Factors Influencing Women's Choice of Mastectomy Versus Breast Conserving Therapy in Early Stage Breast Cancer: A Systematic Review. *Clin Breast Cancer*, 18(4), e539-e554. doi:10.1016/j.clbc.2017.12.013
- Gulino, A., Di Marcotullio, L., & Screpanti, I. (2010). The multiple functions of Numb. *Exp Cell Res*, 316(6), 900-906. doi:10.1016/j.yexcr.2009.11.017
- Guo, W., & Giancotti, F. G. (2004). Integrin signalling during tumour progression. *Nat Rev Mol Cell Biol*, 5(10), 816-826. doi:10.1038/nrm1490
- Gwinn, D. M., Shackelford, D. B., Egan, D. F., Mihaylova, M. M., Mery, A., Vasquez, D. S., . . . Shaw, R. J. (2008). AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell*, 30(2), 214-226. doi:10.1016/j.molcel.2008.03.003
- Haensel, D., Sun, P., MacLean, A. L., Ma, X., Zhou, Y., Stemmler, M. P., . . . Dai, X. (2019). An Ovol2-Zeb1 transcriptional circuit regulates epithelial directional migration and proliferation. *EMBO Rep*, 20(1). doi:10.15252/embr.201846273
- Hapach, L. A., Carey, S. P., Schwager, S. C., Taufalele, P. V., Wang, W., Mosier, J. A., . . . Reinhart-King, C. A. (2021). Phenotypic Heterogeneity and Metastasis of Breast Cancer Cells. *Cancer Res*, 81(13), 3649-3663. doi:10.1158/0008-5472.Can-20-1799
- Harbeck, N., & Gnant, M. (2017). Breast cancer. *Lancet*, 389(10074), 1134-1150. doi:10.1016/s0140-6736(16)31891-8
- Hazan, R. B., Phillips, G. R., Qiao, R. F., Norton, L., & Aaronson, S. A. (2000). Exogenous expression of N-cadherin in breast cancer cells induces cell migration, invasion, and metastasis. *J Cell Biol*, 148(4), 779-790. doi:10.1083/jcb.148.4.779
- Hazan, R. B., Qiao, R., Keren, R., Badano, I., & Suyama, K. (2004). Cadherin switch in tumor progression. *Ann N Y Acad Sci*, 1014, 155-163. doi:10.1196/annals.1294.016
- He, Y., Northey, J. J., Pelletier, A., Kos, Z., Meunier, L., Haibe-Kains, B., . . . Lamarche-Vane, N. (2017). The Cdc42/Rac1 regulator CdGAP is a novel E-cadherin transcriptional co-repressor with Zeb2 in breast cancer. *Oncogene*, 36(24), 3490-3503. doi:10.1038/onc.2016.492
- Hindupur, S. K., Balaji, S. A., Saxena, M., Pandey, S., Sravan, G. S., Heda, N., . . . Rangarajan, A. (2014). Identification of a novel AMPK-PEA15 axis in the anoikis-resistant growth of mammary cells. *Breast Cancer Res*, 16(4), 420. doi:10.1186/s13058-014-0420-z
- Hong, T., Watanabe, K., Ta, C. H., Villarreal-Ponce, A., Nie, Q., & Dai, X. (2015). An Ovol2-Zeb1 Mutual Inhibitory Circuit Governs Bidirectional and Multi-step Transition between Epithelial and Mesenchymal States. *PLoS Comput Biol*, 11(11), e1004569. doi:10.1371/journal.pcbi.1004569
- Horne, H. N., Sherman, M. E., Garcia-Closas, M., Pharoah, P. D., Blows, F. M., Yang, X. R., . . . Figueroa, J. D. (2014). Breast cancer susceptibility risk associations and heterogeneity

- by E-cadherin tumor tissue expression. *Breast Cancer Res Treat*, 143(1), 181-187. doi:10.1007/s10549-013-2771-z
- Hu, M., Xia, M., Chen, X., Lin, Z., Xu, Y., Ma, Y., & Su, L. (2010). MicroRNA-141 regulates Smad interacting protein 1 (SIP1) and inhibits migration and invasion of colorectal cancer cells. *Dig Dis Sci*, 55(8), 2365-2372. doi:10.1007/s10620-009-1008-9
- Hu, X. B., Ouyang, L. Z., He, Y., & Xia, M. Z. (2019). Numb confers to inhibit epithelial mesenchymal transition via β -catenin/Lin28 signaling pathway in breast cancer. *Exp Mol Pathol*, 109, 104262. doi:10.1016/j.yexmp.2019.104262
- Huang, L., Yang, Y., Yang, F., Liu, S., Zhu, Z., Lei, Z., & Guo, J. (2018). Functions of EpCAM in physiological processes and diseases (Review). *Int J Mol Med*, 42(4), 1771-1785. doi:10.3892/ijmm.2018.3764
- Hui, L., Zhang, S., Dong, X., Tian, D., Cui, Z., & Qiu, X. (2013). Prognostic significance of twist and N-cadherin expression in NSCLC. *PLoS One*, 8(4), e62171. doi:10.1371/journal.pone.0062171
- Hynes, R. O. (2002). Integrins: bidirectional, allosteric signaling machines. *Cell*, 110(6), 673-687. doi:10.1016/s0092-8674(02)00971-6
- Hyun, K. A., Koo, G. B., Han, H., Sohn, J., Choi, W., Kim, S. I., . . . Kim, Y. S. (2016). Epithelial-to-mesenchymal transition leads to loss of EpCAM and different physical properties in circulating tumor cells from metastatic breast cancer. *Oncotarget*, 7(17), 24677-24687. doi:10.18632/oncotarget.8250
- Idoux-Gillet, Y., Nassour, M., Lakis, E., Bonini, F., Theillet, C., Du Manoir, S., & Savagner, P. (2018). Slug/Pcad pathway controls epithelial cell dynamics in mammary gland and breast carcinoma. *Oncogene*, 37(5), 578-588. doi:10.1038/onc.2017.355
- Imani, S., Hosseinfard, H., Cheng, J., Wei, C., & Fu, J. (2016). Prognostic Value of EMT-inducing Transcription Factors (EMT-TFs) in Metastatic Breast Cancer: A Systematic Review and Meta-analysis. *Sci Rep*, 6, 28587. doi:10.1038/srep28587
- Jarrard, D. F., Paul, R., van Bokhoven, A., Nguyen, S. H., Bova, G. S., Wheelock, M. J., . . . Isaacs, W. B. (1997). P-Cadherin is a basal cell-specific epithelial marker that is not expressed in prostate cancer. *Clin Cancer Res*, 3(11), 2121-2128.
- Jeon, S. M., & Hay, N. (2015). The double-edged sword of AMPK signaling in cancer and its therapeutic implications. *Arch Pharm Res*, 38(3), 346-357. doi:10.1007/s12272-015-0549-z
- Jie, D., Zhongmin, Z., Guoqing, L., Sheng, L., Yi, Z., Jing, W., & Liang, Z. (2013). Positive expression of LSD1 and negative expression of E-cadherin correlate with metastasis and poor prognosis of colon cancer. *Dig Dis Sci*, 58(6), 1581-1589. doi:10.1007/s10620-012-2552-2
- Jolly, M. K., Boareto, M., Debeb, B. G., Aceto, N., Farach-Carson, M. C., Woodward, W. A., & Levine, H. (2017). Inflammatory breast cancer: a model for investigating cluster-based dissemination. *NPJ Breast Cancer*, 3, 21. doi:10.1038/s41523-017-0023-9
- Jolly, M. K., Boareto, M., Huang, B., Jia, D., Lu, M., Ben-Jacob, E., . . . Levine, H. (2015). Implications of the Hybrid Epithelial/Mesenchymal Phenotype in Metastasis. *Front Oncol*, 5, 155. doi:10.3389/fonc.2015.00155
- Jolly, M. K., Somarelli, J. A., Sheth, M., Biddle, A., Tripathi, S. C., Armstrong, A. J., . . . Levine, H. (2019). Hybrid epithelial/mesenchymal phenotypes promote metastasis and therapy resistance across carcinomas. *Pharmacol Ther*, 194, 161-184. doi:10.1016/j.pharmthera.2018.09.007
- Jolly, M. K., Tripathi, S. C., Jia, D., Mooney, S. M., Celikbas, M., Hanash, S. M., . . . Levine, H. (2016). Stability of the hybrid epithelial/mesenchymal phenotype. *Oncotarget*, 7(19), 27067-27084. doi:10.18632/oncotarget.8166
- Jones, R. G., Plas, D. R., Kubek, S., Buzzai, M., Mu, J., Xu, Y., . . . Thompson, C. B. (2005). AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. *Mol Cell*, 18(3), 283-293. doi:10.1016/j.molcel.2005.03.027

- Jung, C. H., Ro, S. H., Cao, J., Otto, N. M., & Kim, D. H. (2010). mTOR regulation of autophagy. *FEBS Lett*, 584(7), 1287-1295. doi:10.1016/j.febslet.2010.01.017
- Kallergi, G., Konstantinidis, G., Markomanolaki, H., Papadaki, M. A., Mavroudis, D., Stournaras, C., . . . Agelaki, S. (2013). Apoptotic circulating tumor cells in early and metastatic breast cancer patients. *Mol Cancer Ther*, 12(9), 1886-1895. doi:10.1158/1535-7163.Mct-12-1167
- Kapil, U., Bhadoria, A. S., Sareen, N., Singh, P., & Dwivedi, S. N. (2014). Reproductive factors and risk of breast cancer: A Review. *Indian J Cancer*, 51(4), 571-576. doi:10.4103/0019-509x.175345
- Karihtala, P., Auvinen, P., Kauppila, S., Haapasaari, K. M., Jukkola-Vuorinen, A., & Soini, Y. (2013). Vimentin, zeb1 and Sip1 are up-regulated in triple-negative and basal-like breast cancers: association with an aggressive tumour phenotype. *Breast Cancer Res Treat*, 138(1), 81-90. doi:10.1007/s10549-013-2442-0
- Katsura, A., Tamura, Y., Hokari, S., Harada, M., Morikawa, M., Sakurai, T., . . . Koinuma, D. (2017). ZEB1-regulated inflammatory phenotype in breast cancer cells. *Mol Oncol*, 11(9), 1241-1262. doi:10.1002/1878-0261.12098
- Kemper, M., Schiecke, A., Maar, H., Nikulin, S., Poloznikov, A., Galatenko, V., . . . Wicklein, D. (2021). Integrin alpha-V is an important driver in pancreatic adenocarcinoma progression. *J Exp Clin Cancer Res*, 40(1), 214. doi:10.1186/s13046-021-01946-2
- Kim, E. K., Park, J. M., Lim, S., Choi, J. W., Kim, H. S., Seok, H., . . . Suh, P. G. (2011). Activation of AMP-activated protein kinase is essential for lysophosphatidic acid-induced cell migration in ovarian cancer cells. *J Biol Chem*, 286(27), 24036-24045. doi:10.1074/jbc.M110.209908
- Klionsky, D. J., Petroni, G., Amaravadi, R. K., Baehrecke, E. H., Ballabio, A., Boya, P., . . . Pietrocola, F. (2021). Autophagy in major human diseases. *Embo j*, e108863. doi:10.15252/emboj.2021108863
- Ko, J. H., Yang, M. H., Baek, S. H., Nam, D., Jung, S. H., & Ahn, K. S. (2019). Theacrine attenuates epithelial mesenchymal transition in human breast cancer MDA-MB-231 cells. *Phytother Res*, 33(7), 1934-1942. doi:10.1002/ptr.6389
- Koga, F., Kawakami, S., Kumagai, J., Takizawa, T., Ando, N., Arai, G., . . . Kihara, K. (2003). Impaired Delta Np63 expression associates with reduced beta-catenin and aggressive phenotypes of urothelial neoplasms. *Br J Cancer*, 88(5), 740-747. doi:10.1038/sj.bjc.6600764
- Kong, D. H., Kim, Y. K., Kim, M. R., Jang, J. H., & Lee, S. (2018). Emerging Roles of Vascular Cell Adhesion Molecule-1 (VCAM-1) in Immunological Disorders and Cancer. *Int J Mol Sci*, 19(4). doi:10.3390/ijms19041057
- Korsching, E., Packeisen, J., Liedtke, C., Hungermann, D., Wülfing, P., van Diest, P. J., . . . Buerger, H. (2005). The origin of vimentin expression in invasive breast cancer: epithelial-mesenchymal transition, myoepithelial histogenesis or histogenesis from progenitor cells with bilinear differentiation potential? *J Pathol*, 206(4), 451-457. doi:10.1002/path.1797
- Kreutzfeldt, J., Rozeboom, B., Dey, N., & De, P. (2020). The trastuzumab era: current and upcoming targeted HER2+ breast cancer therapies. *Am J Cancer Res*, 10(4), 1045-1067.
- Kröger, C., Afeyan, A., Mraz, J., Eaton, E. N., Reinhardt, F., Khodor, Y. L., . . . Weinberg, R. A. (2019). Acquisition of a hybrid E/M state is essential for tumorigenicity of basal breast cancer cells. *Proc Natl Acad Sci U S A*, 116(15), 7353-7362. doi:10.1073/pnas.1812876116
- Krug, D., Baumann, R., Budach, W., Dunst, J., Feyer, P., Fietkau, R., . . . Sauer, R. (2017). Current controversies in radiotherapy for breast cancer. *Radiat Oncol*, 12(1), 25. doi:10.1186/s13014-017-0766-3

- Kryczek, I., Liu, S., Roh, M., Vatan, L., Szeliga, W., Wei, S., . . . Zou, W. (2012). Expression of aldehyde dehydrogenase and CD133 defines ovarian cancer stem cells. *Int J Cancer*, 130(1), 29-39. doi:10.1002/ijc.25967
- Kudo-Saito, C., Shirako, H., Takeuchi, T., & Kawakami, Y. (2009). Cancer metastasis is accelerated through immunosuppression during Snail-induced EMT of cancer cells. *Cancer Cell*, 15(3), 195-206. doi:10.1016/j.ccr.2009.01.023
- Lakhtakia, R., Aljarrah, A., Furruk, M., & Ganguly, S. S. (2017). Epithelial Mesenchymal Transition (EMT) in Metastatic Breast Cancer in Omani Women. *Cancer Microenviron*, 10(1-3), 25-37. doi:10.1007/s12307-017-0194-9
- Lamb, C. A., Vanzulli, S. I., & Lanari, C. (2019). Hormone receptors in breast cancer: more than estrogen receptors. *Medicina (B Aires)*, 79(Spec 6/1), 540-545.
- Lambert, A. W., Pattabiraman, D. R., & Weinberg, R. A. (2017). Emerging Biological Principles of Metastasis. *Cell*, 168(4), 670-691. doi:10.1016/j.cell.2016.11.037
- Lau, A., Villeneuve, N. F., Sun, Z., Wong, P. K., & Zhang, D. D. (2008). Dual roles of Nrf2 in cancer. *Pharmacol Res*, 58(5-6), 262-270. doi:10.1016/j.phrs.2008.09.003
- Leckband, D., & Sivasankar, S. (2012). Cadherin recognition and adhesion. *Curr Opin Cell Biol*, 24(5), 620-627. doi:10.1016/j.ceb.2012.05.014
- Lee, K., & Nelson, C. M. (2012). New insights into the regulation of epithelial-mesenchymal transition and tissue fibrosis. *Int Rev Cell Mol Biol*, 294, 171-221. doi:10.1016/b978-0-12-394305-7.00004-5
- Lee, Y. J., Bernstock, J. D., Klimanis, D., & Hallenbeck, J. M. (2018). Akt Protein Kinase, miR-200/miR-182 Expression and Epithelial-Mesenchymal Transition Proteins in Hibernating Ground Squirrels. *Front Mol Neurosci*, 11, 22. doi:10.3389/fnmol.2018.00022
- Lehman, H. L., Dashner, E. J., Lucey, M., Vermeulen, P., Dirix, L., Van Laere, S., & van Golen, K. L. (2013). Modeling and characterization of inflammatory breast cancer emboli grown in vitro. *Int J Cancer*, 132(10), 2283-2294. doi:10.1002/ijc.27928
- Lei, Y., Huang, K., Gao, C., Lau, Q. C., Pan, H., Xie, K., . . . Wei, Y. (2011). Proteomics identification of ITGB3 as a key regulator in reactive oxygen species-induced migration and invasion of colorectal cancer cells. *Mol Cell Proteomics*, 10(10), M110.005397. doi:10.1074/mcp.M110.005397
- Li, C., Wang, J., Kong, J., Tang, J., Wu, Y., Xu, E., . . . Lai, M. (2016). GDF15 promotes EMT and metastasis in colorectal cancer. *Oncotarget*, 7(1), 860-872. doi:10.18632/oncotarget.6205
- Li, X. L., Liu, L., Li, D. D., He, Y. P., Guo, L. H., Sun, L. P., . . . Zhang, X. P. (2017). Integrin $\beta 4$ promotes cell invasion and epithelial-mesenchymal transition through the modulation of Slug expression in hepatocellular carcinoma. *Sci Rep*, 7, 40464. doi:10.1038/srep40464
- Liang, J., Shao, S. H., Xu, Z. X., Hennessy, B., Ding, Z., Larrea, M., . . . Mills, G. B. (2007). The energy sensing LKB1-AMPK pathway regulates p27(kip1) phosphorylation mediating the decision to enter autophagy or apoptosis. *Nat Cell Biol*, 9(2), 218-224. doi:10.1038/ncb1537
- Liao, T. T., & Yang, M. H. (2020). Hybrid Epithelial/Mesenchymal State in Cancer Metastasis: Clinical Significance and Regulatory Mechanisms. *Cells*, 9(3). doi:10.3390/cells9030623
- Lindsay, J., McDade, S. S., Pickard, A., McCloskey, K. D., & McCance, D. J. (2011). Role of DeltaNp63gamma in epithelial to mesenchymal transition. *J Biol Chem*, 286(5), 3915-3924. doi:10.1074/jbc.M110.162511
- Liu, C. Y., Lin, H. H., Tang, M. J., & Wang, Y. K. (2015). Vimentin contributes to epithelial-mesenchymal transition cancer cell mechanics by mediating cytoskeletal organization and focal adhesion maturation. *Oncotarget*, 6(18), 15966-15983. doi:10.18632/oncotarget.3862

- Liu, N., Yu, Q., Liu, T. J., Gebreamlak, E. P., Wang, S. L., Zhang, R. J., . . . Niu, Y. (2012). P-cadherin expression and basal-like subtype in breast cancers. *Med Oncol*, 29(4), 2606-2612. doi:10.1007/s12032-012-0218-8
- Liu, X., Li, J., Cadilha, B. L., Markota, A., Voigt, C., Huang, Z., . . . Wang, H. (2019). Epithelial-type systemic breast carcinoma cells with a restricted mesenchymal transition are a major source of metastasis. *Sci Adv*, 5(6), eaav4275. doi:10.1126/sciadv.aav4275
- Loeser, H., Scholz, M., Fuchs, H., Essakly, A., Damanakis, A. I., Zander, T., . . . Gebauer, F. (2020). Integrin alpha V (ITGAV) expression in esophageal adenocarcinoma is associated with shortened overall-survival. *Sci Rep*, 10(1), 18411. doi:10.1038/s41598-020-75085-7
- Logsdon, C. D., Simeone, D. M., Binkley, C., Arumugam, T., Greenson, J. K., Giordano, T. J., . . . Hanash, S. (2003). Molecular profiling of pancreatic adenocarcinoma and chronic pancreatitis identifies multiple genes differentially regulated in pancreatic cancer. *Cancer Res*, 63(10), 2649-2657.
- Louderbough, J. M., & Schroeder, J. A. (2011). Understanding the dual nature of CD44 in breast cancer progression. *Mol Cancer Res*, 9(12), 1573-1586. doi:10.1158/1541-7786.Mcr-11-0156
- Low, S. K., Zembutsu, H., & Nakamura, Y. (2018). Breast cancer: The translation of big genomic data to cancer precision medicine. *Cancer Sci*, 109(3), 497-506. doi:10.1111/cas.13463
- Lowery, A. J., Kell, M. R., Glynn, R. W., Kerin, M. J., & Sweeney, K. J. (2012). Locoregional recurrence after breast cancer surgery: a systematic review by receptor phenotype. *Breast Cancer Res Treat*, 133(3), 831-841. doi:10.1007/s10549-011-1891-6
- Lu, S., Lee, J., Revelo, M., Wang, X., Lu, S., & Dong, Z. (2007). Smad3 is overexpressed in advanced human prostate cancer and necessary for progressive growth of prostate cancer cells in nude mice. *Clin Cancer Res*, 13(19), 5692-5702. doi:10.1158/1078-0432.Ccr-07-1078
- Lu, S., Simin, K., Khan, A., & Mercurio, A. M. (2008). Analysis of integrin beta4 expression in human breast cancer: association with basal-like tumors and prognostic significance. *Clin Cancer Res*, 14(4), 1050-1058. doi:10.1158/1078-0432.Ccr-07-4116
- Lu, X., Mu, E., Wei, Y., Riethdorf, S., Yang, Q., Yuan, M., . . . Kang, Y. (2011). VCAM-1 promotes osteolytic expansion of indolent bone micrometastasis of breast cancer by engaging $\alpha\beta 1$ -positive osteoclast progenitors. *Cancer Cell*, 20(6), 701-714. doi:10.1016/j.ccr.2011.11.002
- Lundgren, K., Nordenskjöld, B., & Landberg, G. (2009). Hypoxia, Snail and incomplete epithelial-mesenchymal transition in breast cancer. *Br J Cancer*, 101(10), 1769-1781. doi:10.1038/sj.bjc.6605369
- Luo, Y., Yu, T., Zhang, Q., Fu, Q., Hu, Y., Xiang, M., . . . Shi, H. (2018). Upregulated N-cadherin expression is associated with poor prognosis in epithelial-derived solid tumours: A meta-analysis. *Eur J Clin Invest*, 48(4). doi:10.1111/eci.12903
- Lyons, J. A., & Sherertz, T. (2014). Postmastectomy radiation therapy. *Curr Oncol Rep*, 16(1), 361. doi:10.1007/s11912-013-0361-1
- Mandal, M., Myers, J. N., Lippman, S. M., Johnson, F. M., Williams, M. D., Rayala, S., . . . El-Naggar, A. K. (2008). Epithelial to mesenchymal transition in head and neck squamous carcinoma: association of Src activation with E-cadherin down-regulation, vimentin expression, and aggressive tumor features. *Cancer*, 112(9), 2088-2100. doi:10.1002/cncr.23410
- Mandeville, J. A., Silva Neto, B., Vanni, A. J., Smith, G. L., Rieger-Christ, K. M., Zeheb, R., . . . Summerhayes, I. C. (2008). P-cadherin as a prognostic indicator and a modulator of migratory behaviour in bladder carcinoma cells. *BJU Int*, 102(11), 1707-1714. doi:10.1111/j.1464-410X.2008.08115.x
- Mani, S. A., Guo, W., Liao, M. J., Eaton, E. N., Ayyanan, A., Zhou, A. Y., . . . Weinberg, R. A. (2008). The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell*, 133(4), 704-715. doi:10.1016/j.cell.2008.03.027

- Marcucci, F., Ghezzi, P., & Rumio, C. (2017). The role of autophagy in the cross-talk between epithelial-mesenchymal transitioned tumor cells and cancer stem-like cells. *Mol Cancer*, 16(1), 3. doi:10.1186/s12943-016-0573-8
- Martin, T. A., Mansel, R. E., & Jiang, W. G. (2010). Loss of occludin leads to the progression of human breast cancer. *Int J Mol Med*, 26(5), 723-734. doi:10.3892/ijmm_00000519
- Martino, F., Perestrelo, A. R., Vinarský, V., Pagliari, S., & Forte, G. (2018). Cellular Mechanotransduction: From Tension to Function. *Front Physiol*, 9, 824. doi:10.3389/fphys.2018.00824
- Masuda, T., Ueo, H., Kai, Y., Noda, M., Hu, Q., Sato, K., . . . Ueo, H. (2020). N-Cadherin mRNA Levels in Peripheral Blood Could Be a Potential Indicator of New Metastases in Breast Cancer: A Pilot Study. *Int J Mol Sci*, 21(2). doi:10.3390/ijms21020511
- Masugi, Y., Yamazaki, K., Emoto, K., Effendi, K., Tsujikawa, H., Kitago, M., . . . Sakamoto, M. (2015). Upregulation of integrin $\beta 4$ promotes epithelial-mesenchymal transition and is a novel prognostic marker in pancreatic ductal adenocarcinoma. *Lab Invest*, 95(3), 308-319. doi:10.1038/labinvest.2014.166
- McNeill, B., Vulesevic, B., Ostojic, A., Ruel, M., & Suuronen, E. J. (2015). Collagen matrix-induced expression of integrin $\alpha V\beta 3$ in circulating angiogenic cells can be targeted by matricellular protein CCN1 to enhance their function. *Faseb j*, 29(4), 1198-1207. doi:10.1096/fj.14-261586
- Mecca, C., Giambanco, I., Bruscoli, S., Bereshchenko, O., Fioretti, B., Riccardi, C., . . . Arcuri, C. (2018). PP242 Counteracts Glioblastoma Cell Proliferation, Migration, Invasiveness and Stemness Properties by Inhibiting mTORC2/AKT. *Front Cell Neurosci*, 12, 99. doi:10.3389/fncel.2018.00099
- Mejlvang, J., Kriaevska, M., Vandewalle, C., Chernova, T., Sayan, A. E., Berx, G., . . . Tulchinsky, E. (2007). Direct repression of cyclin D1 by SIP1 attenuates cell cycle progression in cells undergoing an epithelial mesenchymal transition. *Mol Biol Cell*, 18(11), 4615-4624. doi:10.1091/mbc.e07-05-0406
- Memmi, E. M., Sanarico, A. G., Giacobbe, A., Peschiaroli, A., Frezza, V., Cicalese, A., . . . Bernassola, F. (2015). p63 Sustains self-renewal of mammary cancer stem cells through regulation of Sonic Hedgehog signaling. *Proc Natl Acad Sci U S A*, 112(11), 3499-3504. doi:10.1073/pnas.1500762112
- Mendez, M. G., Kojima, S., & Goldman, R. D. (2010). Vimentin induces changes in cell shape, motility, and adhesion during the epithelial to mesenchymal transition. *Faseb j*, 24(6), 1838-1851. doi:10.1096/fj.09-151639
- Menegon, S., Columbano, A., & Giordano, S. (2016). The Dual Roles of NRF2 in Cancer. *Trends Mol Med*, 22(7), 578-593. doi:10.1016/j.molmed.2016.05.002
- Micalizzi, D. S., Farabaugh, S. M., & Ford, H. L. (2010). Epithelial-mesenchymal transition in cancer: parallels between normal development and tumor progression. *J Mammary Gland Biol Neoplasia*, 15(2), 117-134. doi:10.1007/s10911-010-9178-9
- Mlacki, M., Kikulska, A., Krzywinska, E., Pawlak, M., & Wilanowski, T. (2015). Recent discoveries concerning the involvement of transcription factors from the Grainyhead-like family in cancer. *Exp Biol Med (Maywood)*, 240(11), 1396-1401. doi:10.1177/1535370215588924
- Moo, T. A., Sanford, R., Dang, C., & Morrow, M. (2018). Overview of Breast Cancer Therapy. *PET Clin*, 13(3), 339-354. doi:10.1016/j.cpet.2018.02.006
- Mooney, S. M., Talebian, V., Jolly, M. K., Jia, D., Gromala, M., Levine, H., & McConkey, B. J. (2017). The GRHL2/ZEB Feedback Loop-A Key Axis in the Regulation of EMT in Breast Cancer. *J Cell Biochem*, 118(9), 2559-2570. doi:10.1002/jcb.25974
- Morris, L. G., & Chan, T. A. (2015). Therapeutic targeting of tumor suppressor genes. *Cancer*, 121(9), 1357-1368. doi:10.1002/cncr.29140
- Motohashi, H., & Yamamoto, M. (2004). Nrf2-Keap1 defines a physiologically important stress response mechanism. *Trends Mol Med*, 10(11), 549-557. doi:10.1016/j.molmed.2004.09.003

- Müller, V., Stahmann, N., Riethdorf, S., Rau, T., Zabel, T., Goetz, A., . . . Pantel, K. (2005). Circulating tumor cells in breast cancer: correlation to bone marrow micrometastases, heterogeneous response to systemic therapy and low proliferative activity. *Clin Cancer Res*, 11(10), 3678-3685. doi:10.1158/1078-0432.Ccr-04-2469
- Muramaki, M., Miyake, H., Terakawa, T., Kusuda, Y., & Fujisawa, M. (2011). Expression profile of E-cadherin and N-cadherin in urothelial carcinoma of the upper urinary tract is associated with disease recurrence in patients undergoing nephroureterectomy. *Urology*, 78(6), 1443.e1447-1412. doi:10.1016/j.urology.2011.07.1388
- Na, T. Y., Schecterson, L., Mendonsa, A. M., & Gumbiner, B. M. (2020). The functional activity of E-cadherin controls tumor cell metastasis at multiple steps. *Proc Natl Acad Sci U S A*, 117(11), 5931-5937. doi:10.1073/pnas.1918167117
- Neve, R. M., Chin, K., Fridlyand, J., Yeh, J., Baehner, F. L., Fevr, T., . . . Gray, J. W. (2006). A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. *Cancer Cell*, 10(6), 515-527. doi:10.1016/j.ccr.2006.10.008
- Ng, T. L., Leprivier, G., Robertson, M. D., Chow, C., Martin, M. J., Laderoute, K. R., . . . Sorensen, P. H. (2012). The AMPK stress response pathway mediates anoikis resistance through inhibition of mTOR and suppression of protein synthesis. *Cell Death Differ*, 19(3), 501-510. doi:10.1038/cdd.2011.119
- Nieto, M. A. (2013). Epithelial plasticity: a common theme in embryonic and cancer cells. *Science*, 342(6159), 1234850. doi:10.1126/science.1234850
- Nieto, M. A., Huang, R. Y., Jackson, R. A., & Thiery, J. P. (2016). EMT: 2016. *Cell*, 166(1), 21-45. doi:10.1016/j.cell.2016.06.028
- Nishimura, T., & Kaibuchi, K. (2007). Numb controls integrin endocytosis for directional cell migration with aPKC and PAR-3. *Dev Cell*, 13(1), 15-28. doi:10.1016/j.devcel.2007.05.003
- Niu, J., & Li, Z. (2017). The roles of integrin $\alpha\beta 6$ in cancer. *Cancer Lett*, 403, 128-137. doi:10.1016/j.canlet.2017.06.012
- Nollet, F., Kools, P., & van Roy, F. (2000). Phylogenetic analysis of the cadherin superfamily allows identification of six major subfamilies besides several solitary members. *J Mol Biol*, 299(3), 551-572. doi:10.1006/jmbi.2000.3777
- Nose, A., & Takeichi, M. (1986). A novel cadherin cell adhesion molecule: its expression patterns associated with implantation and organogenesis of mouse embryos. *J Cell Biol*, 103(6 Pt 2), 2649-2658. doi:10.1083/jcb.103.6.2649
- Nowicki, A., Sporny, S., & Duda-Szymańska, J. (2012). β -catenin as a prognostic factor for prostate cancer (PCa). *Cent European J Urol*, 65(3), 119-123. doi:10.5173/ceju.2012.03.art4
- Odero-Marrah, V., Hawsawi, O., Henderson, V., & Sweeney, J. (2018). Epithelial-Mesenchymal Transition (EMT) and Prostate Cancer. *Adv Exp Med Biol*, 1095, 101-110. doi:10.1007/978-3-319-95693-0_6
- Oh, D. Y., & Bang, Y. J. (2020). HER2-targeted therapies - a role beyond breast cancer. *Nat Rev Clin Oncol*, 17(1), 33-48. doi:10.1038/s41571-019-0268-3
- Ohkubo, T., & Ozawa, M. (2004). The transcription factor Snail downregulates the tight junction components independently of E-cadherin downregulation. *J Cell Sci*, 117(Pt 9), 1675-1685. doi:10.1242/jcs.01004
- Osorio, L. A., Farfán, N. M., Castellón, E. A., & Contreras, H. R. (2016). SNAIL transcription factor increases the motility and invasive capacity of prostate cancer cells. *Mol Med Rep*, 13(1), 778-786. doi:10.3892/mmr.2015.4585
- Padmanaban, V., Krol, I., Suhail, Y., Szczerba, B. M., Aceto, N., Bader, J. S., & Ewald, A. J. (2019). E-cadherin is required for metastasis in multiple models of breast cancer. *Nature*, 573(7774), 439-444. doi:10.1038/s41586-019-1526-3
- Pal, M., Chen, H., Lee, B. H., Lee, J. Y. H., Yip, Y. S., Tan, N. S., & Tan, L. P. (2019). Epithelial-mesenchymal transition of cancer cells using bioengineered hybrid scaffold composed

- of hydrogel/3D-fibrous framework. *Sci Rep*, 9(1), 8997. doi:10.1038/s41598-019-45384-9
- Palacios, J., Benito, N., Pizarro, A., Suárez, A., Espada, J., Cano, A., & Gamallo, C. (1995). Anomalous expression of P-cadherin in breast carcinoma. Correlation with E-cadherin expression and pathological features. *Am J Pathol*, 146(3), 605-612.
- Paredes, J., Albergaria, A., Oliveira, J. T., Jerónimo, C., Milanezi, F., & Schmitt, F. C. (2005). P-cadherin overexpression is an indicator of clinical outcome in invasive breast carcinomas and is associated with CDH3 promoter hypomethylation. *Clin Cancer Res*, 11(16), 5869-5877. doi:10.1158/1078-0432.Ccr-05-0059
- Paredes, J., Correia, A. L., Ribeiro, A. S., Albergaria, A., Milanezi, F., & Schmitt, F. C. (2007). P-cadherin expression in breast cancer: a review. *Breast Cancer Res*, 9(5), 214. doi:10.1186/bcr1774
- Paredes, J., Correia, A. L., Ribeiro, A. S., Milanezi, F., Cameselle-Teijeiro, J., & Schmitt, F. C. (2008). Breast carcinomas that co-express E- and P-cadherin are associated with p120-catenin cytoplasmic localisation and poor patient survival. *J Clin Pathol*, 61(7), 856-862. doi:10.1136/jcp.2007.052704
- Paredes, J., Milanezi, F., Viegas, L., Amendoeira, I., & Schmitt, F. (2002). P-cadherin expression is associated with high-grade ductal carcinoma in situ of the breast. *Virchows Arch*, 440(1), 16-21. doi:10.1007/s004280100487
- Paredes, J., Stove, C., Stove, V., Milanezi, F., Van Marck, V., Derycke, L., . . . Schmitt, F. (2004). P-cadherin is up-regulated by the antiestrogen ICI 182,780 and promotes invasion of human breast cancer cells. *Cancer Res*, 64(22), 8309-8317. doi:10.1158/0008-5472.Can-04-0795
- Parvani, J. G., Gujrati, M. D., Mack, M. A., Schiemann, W. P., & Lu, Z. R. (2015). Silencing $\beta 3$ Integrin by Targeted ECO/siRNA Nanoparticles Inhibits EMT and Metastasis of Triple-Negative Breast Cancer. *Cancer Res*, 75(11), 2316-2325. doi:10.1158/0008-5472.Can-14-3485
- Pasani, S., Sahoo, S., & Jolly, M. K. (2020). Hybrid E/M Phenotype(s) and Stemness: A Mechanistic Connection Embedded in Network Topology. *J Clin Med*, 10(1). doi:10.3390/jcm10010060
- Pastushenko, I., & Blanpain, C. (2019). EMT Transition States during Tumor Progression and Metastasis. *Trends Cell Biol*, 29(3), 212-226. doi:10.1016/j.tcb.2018.12.001
- Pastushenko, I., Brisebarre, A., Sifrim, A., Fioramonti, M., Revenco, T., Boumahdi, S., . . . Blanpain, C. (2018). Identification of the tumour transition states occurring during EMT. *Nature*, 556(7702), 463-468. doi:10.1038/s41586-018-0040-3
- Patturajan, M., Nomoto, S., Sommer, M., Fomenkov, A., Hibi, K., Zangen, R., . . . Sidransky, D. (2002). DeltaNp63 induces beta-catenin nuclear accumulation and signaling. *Cancer Cell*, 1(4), 369-379. doi:10.1016/s1535-6108(02)00057-0
- Pece, S., Confalonieri, S., P, R. R., & Di Fiore, P. P. (2011). NUMB-ing down cancer by more than just a NOTCH. *Biochim Biophys Acta*, 1815(1), 26-43. doi:10.1016/j.bbcan.2010.10.001
- Pece, S., Serresi, M., Santolini, E., Capra, M., Hulleman, E., Galimberti, V., . . . Di Fiore, P. P. (2004). Loss of negative regulation by Numb over Notch is relevant to human breast carcinogenesis. *J Cell Biol*, 167(2), 215-221. doi:10.1083/jcb.200406140
- Peinado, H., Olmeda, D., & Cano, A. (2007). Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nat Rev Cancer*, 7(6), 415-428. doi:10.1038/nrc2131
- Peralta Soler, A., Knudsen, K. A., Salazar, H., Han, A. C., & Keshgegian, A. A. (1999). P-cadherin expression in breast carcinoma indicates poor survival. *Cancer*, 86(7), 1263-1272. doi:10.1002/(sici)1097-0142(19991001)86:7<1263::aid-cnrc23>3.3.co;2-u
- Perou, C. M., Sørli, T., Eisen, M. B., van de Rijn, M., Jeffrey, S. S., Rees, C. A., . . . Botstein, D. (2000). Molecular portraits of human breast tumours. *Nature*, 406(6797), 747-752. doi:10.1038/35021093

- Petri, B. J., & Klinge, C. M. (2020). Regulation of breast cancer metastasis signaling by miRNAs. *Cancer Metastasis Rev*, 39(3), 837-886. doi:10.1007/s10555-020-09905-7
- Pilewskie, M., & Morrow, M. (2017). Axillary Nodal Management Following Neoadjuvant Chemotherapy: A Review. *JAMA Oncol*, 3(4), 549-555. doi:10.1001/jamaoncol.2016.4163
- Piñeiro, R., Martínez-Pena, I., & López-López, R. (2020). Relevance of CTC Clusters in Breast Cancer Metastasis. *Adv Exp Med Biol*, 1220, 93-115. doi:10.1007/978-3-030-35805-1_7
- Plutoni, C., Bazellieres, E., & Gauthier-Rouvière, C. (2016). P-cadherin-mediated Rho GTPase regulation during collective cell migration. *Small GTPases*, 7(3), 156-163. doi:10.1080/21541248.2016.1173772
- Ponti, D., Costa, A., Zaffaroni, N., Pratesi, G., Petrangolini, G., Coradini, D., . . . Daidone, M. G. (2005). Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. *Cancer Res*, 65(13), 5506-5511. doi:10.1158/0008-5472.Can-05-0626
- Prasad, C. P., Chaurasiya, S. K., Guilmain, W., & Andersson, T. (2016). WNT5A signaling impairs breast cancer cell migration and invasion via mechanisms independent of the epithelial-mesenchymal transition. *J Exp Clin Cancer Res*, 35(1), 144. doi:10.1186/s13046-016-0421-0
- Prat, A., Pineda, E., Adamo, B., Galván, P., Fernández, A., Gaba, L., . . . Muñoz, M. (2015). Clinical implications of the intrinsic molecular subtypes of breast cancer. *Breast*, 24 Suppl 2, S26-35. doi:10.1016/j.breast.2015.07.008
- Prince, M. E., Sivanandan, R., Kaczorowski, A., Wolf, G. T., Kaplan, M. J., Dalerba, P., . . . Ailles, L. E. (2007). Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proc Natl Acad Sci U S A*, 104(3), 973-978. doi:10.1073/pnas.0610117104
- Provenzano, E., Ulaner, G. A., & Chin, S. F. (2018). Molecular Classification of Breast Cancer. *PET Clin*, 13(3), 325-338. doi:10.1016/j.cpet.2018.02.004
- Qi, R., Lin, J., Chen, S., Jiang, J., Zhang, X., Yao, B., . . . Guo, Q. (2020). Breast cancer prognosis and P-cadherin expression: systematic review and study-level meta-analysis. *BMJ Support Palliat Care*. doi:10.1136/bmjspcare-2020-002204
- Qian, J., Liu, H., Chen, W., Wen, K., Lu, W., Huang, C., & Fu, Z. (2013). Knockdown of Slug by RNAi inhibits the proliferation and invasion of HCT116 colorectal cancer cells. *Mol Med Rep*, 8(4), 1055-1059. doi:10.3892/mmr.2013.1604
- Qin, Q., Xu, Y., He, T., Qin, C., & Xu, J. (2012). Normal and disease-related biological functions of Twist1 and underlying molecular mechanisms. *Cell Res*, 22(1), 90-106. doi:10.1038/cr.2011.144
- Radecka, B., & Litwiniuk, M. (2016). Breast cancer in young women. *Ginek Pol*, 87(9), 659-663. doi:10.5603/gp.2016.0062
- Ratovitski, E. A., Patturajan, M., Hibi, K., Trink, B., Yamaguchi, K., & Sidransky, D. (2001). p53 associates with and targets Delta Np63 into a protein degradation pathway. *Proc Natl Acad Sci U S A*, 98(4), 1817-1822. doi:10.1073/pnas.98.4.1817
- Recht, A., & Solin, L. J. (2011). Breast-conserving surgery and radiotherapy in early-stage breast cancer: the importance of local control. *Semin Radiat Oncol*, 21(1), 3-9. doi:10.1016/j.semradonc.2010.08.001
- Rennstam, K., McMichael, N., Berglund, P., Honeth, G., Hegardt, C., Rydén, L., . . . Hedenfalk, I. (2010). Numb protein expression correlates with a basal-like phenotype and cancer stem cell markers in primary breast cancer. *Breast Cancer Res Treat*, 122(2), 315-324. doi:10.1007/s10549-009-0568-x
- Rezaei, M., Friedrich, K., Wielockx, B., Kuzmanov, A., Kettelhake, A., Labelle, M., . . . Breier, G. (2012). Interplay between neural-cadherin and vascular endothelial-cadherin in breast cancer progression. *Breast Cancer Res*, 14(6), R154. doi:10.1186/bcr3367

- Ribeiro, A. S., Albergaria, A., Sousa, B., Correia, A. L., Bracke, M., Seruca, R., . . . Paredes, J. (2010). Extracellular cleavage and shedding of P-cadherin: a mechanism underlying the invasive behaviour of breast cancer cells. *Oncogene*, 29(3), 392-402. doi:10.1038/onc.2009.338
- Ribeiro, A. S., Carvalho, F. A., Figueiredo, J., Carvalho, R., Mestre, T., Monteiro, J., . . . Paredes, J. (2016). Atomic force microscopy and graph analysis to study the P-cadherin/SFK mechanotransduction signalling in breast cancer cells. *Nanoscale*, 8(46), 19390-19401. doi:10.1039/c6nr04465d
- Ribeiro, A. S., Nobre, A. R., Mendes, N., Almeida, J., Vieira, A. F., Sousa, B., . . . Paredes, J. (2018). SRC inhibition prevents P-cadherin mediated signaling and function in basal-like breast cancer cells. *Cell Commun Signal*, 16(1), 75. doi:10.1186/s12964-018-0286-2
- Ribeiro, A. S., & Paredes, J. (2014). P-Cadherin Linking Breast Cancer Stem Cells and Invasion: A Promising Marker to Identify an "Intermediate/Metastable" EMT State. *Front Oncol*, 4, 371. doi:10.3389/fonc.2014.00371
- Ribeiro, A. S., Sousa, B., Carreto, L., Mendes, N., Nobre, A. R., Ricardo, S., . . . Paredes, J. (2013). P-cadherin functional role is dependent on E-cadherin cellular context: a proof of concept using the breast cancer model. *J Pathol*, 229(5), 705-718. doi:10.1002/path.4143
- Ricardo, S., Vieira, A. F., Gerhard, R., Leitão, D., Pinto, R., Cameselle-Teijeiro, J. F., . . . Paredes, J. (2011). Breast cancer stem cell markers CD44, CD24 and ALDH1: expression distribution within intrinsic molecular subtype. *J Clin Pathol*, 64(11), 937-946. doi:10.1136/jcp.2011.090456
- Root, A. R., Cao, W., Li, B., LaPan, P., Meade, C., Sanford, J., . . . Bloom, L. (2016). Development of PF-06671008, a Highly Potent Anti-P-cadherin/Anti-CD3 Bispecific DART Molecule with Extended Half-Life for the Treatment of Cancer. *Antibodies (Basel)*, 5(1). doi:10.3390/antib5010006
- Rosivatz, E., Becker, I., Specht, K., Fricke, E., Luber, B., Busch, R., . . . Becker, K. F. (2002). Differential expression of the epithelial-mesenchymal transition regulators snail, SIP1, and twist in gastric cancer. *Am J Pathol*, 161(5), 1881-1891. doi:10.1016/s0002-9440(10)64464-1
- Rosso, M., Lapyckyj, L., Amiano, N., Besso, M. J., Sánchez, M., Chuluyan, E., & Vazquez-Levin, M. H. (2014). Secretory Leukocyte Protease Inhibitor (SLPI) expression downregulates E-cadherin, induces β -catenin re-localisation and triggers apoptosis-related events in breast cancer cells. *Biol Cell*, 106(9), 308-322. doi:10.1111/boc.201300075
- Roulot, A., Héquet, D., Guinebretière, J. M., Vincent-Salomon, A., Lerebours, F., Dubot, C., & Rouzier, R. (2016). Tumoral heterogeneity of breast cancer. *Ann Biol Clin (Paris)*, 74(6), 653-660. doi:10.1684/abc.2016.1192
- Saadatmand, S., de Kruijf, E. M., Sajet, A., Dekker-Ensink, N. G., van Nes, J. G., Putter, H., . . . Kuppen, P. J. (2013). Expression of cell adhesion molecules and prognosis in breast cancer. *Br J Surg*, 100(2), 252-260. doi:10.1002/bjs.8980
- Saitoh, M. (2018). Involvement of partial EMT in cancer progression. *J Biochem*, 164(4), 257-264. doi:10.1093/jb/mvy047
- Satelli, A., & Li, S. (2011). Vimentin in cancer and its potential as a molecular target for cancer therapy. *Cell Mol Life Sci*, 68(18), 3033-3046. doi:10.1007/s00018-011-0735-1
- Sathyanarayanan, A., Chandrasekaran, K. S., & Karunakaran, D. (2017). microRNA-145 modulates epithelial-mesenchymal transition and suppresses proliferation, migration and invasion by targeting SIP1 in human cervical cancer cells. *Cell Oncol (Dordr)*, 40(2), 119-131. doi:10.1007/s13402-016-0307-3
- Saxena, K., Srikrishnan, S., Celia-Terrassa, T., & Jolly, M. K. (2020). OVOL1/2: Drivers of Epithelial Differentiation in Development, Disease, and Reprogramming. *Cells Tissues Organs*, 1-10. doi:10.1159/000511383
- Saxena, M., Balaji, S. A., Deshpande, N., Ranganathan, S., Pillai, D. M., Hindupur, S. K., & Rangarajan, A. (2018). AMP-activated protein kinase promotes epithelial-mesenchymal

- transition in cancer cells through Twist1 upregulation. *J Cell Sci*, 131(14). doi:10.1242/jcs.208314
- Seyfried, T. N., & Huysentruyt, L. C. (2013). On the origin of cancer metastasis. *Crit Rev Oncog*, 18(1-2), 43-73. doi:10.1615/critrevoncog.v18.i1-2.40
- Sharma, R., Sharma, R., Khaket, T. P., Dutta, C., Chakraborty, B., & Mukherjee, T. K. (2017). Breast cancer metastasis: Putative therapeutic role of vascular cell adhesion molecule-1. *Cell Oncol (Dordr)*, 40(3), 199-208. doi:10.1007/s13402-017-0324-x
- Sheng, Q., D'Alessio, J. A., Menezes, D. L., Karim, C., Tang, Y., Tam, A., . . . Abrams, T. J. (2021). PCA062, a P-cadherin Targeting Antibody-Drug Conjugate, Displays Potent Antitumor Activity Against P-cadherin-expressing Malignancies. *Mol Cancer Ther*, 20(7), 1270-1282. doi:10.1158/1535-7163.Mct-20-0708
- Shi, Y., Zhang, J., Liu, M., Huang, Y., & Yin, L. (2019). SMAD3 inducing the transcription of STYK1 to promote the EMT process and improve the tolerance of ovarian carcinoma cells to paclitaxel. *J Cell Biochem*, 120(6), 10796-10811. doi:10.1002/jcb.28371
- Shibue, T., & Weinberg, R. A. (2017). EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. *Nat Rev Clin Oncol*, 14(10), 611-629. doi:10.1038/nrclinonc.2017.44
- Shimomura, Y., Wajid, M., Shapiro, L., & Christiano, A. M. (2008). P-cadherin is a p63 target gene with a crucial role in the developing human limb bud and hair follicle. *Development*, 135(4), 743-753. doi:10.1242/dev.006718
- Shimoyama, Y., Yoshida, T., Terada, M., Shimosato, Y., Abe, O., & Hirohashi, S. (1989). Molecular cloning of a human Ca²⁺-dependent cell-cell adhesion molecule homologous to mouse placental cadherin: its low expression in human placental tissues. *J Cell Biol*, 109(4 Pt 1), 1787-1794. doi:10.1083/jcb.109.4.1787
- Si, W., Huang, W., Zheng, Y., Yang, Y., Liu, X., Shan, L., . . . Shang, Y. (2015). Dysfunction of the Reciprocal Feedback Loop between GATA3- and ZEB2-Nucleated Repression Programs Contributes to Breast Cancer Metastasis. *Cancer Cell*, 27(6), 822-836. doi:10.1016/j.ccell.2015.04.011
- Sinha, D., Saha, P., Samanta, A., & Bishayee, A. (2020). Emerging Concepts of Hybrid Epithelial-to-Mesenchymal Transition in Cancer Progression. *Biomolecules*, 10(11). doi:10.3390/biom10111561
- Slade, D. (2020). PARP and PARG inhibitors in cancer treatment. *Genes Dev*, 34(5-6), 360-394. doi:10.1101/gad.334516.119
- Soler, A., Cayrefourcq, L., Mazard, T., Babayan, A., Lamy, P. J., Assou, S., . . . Alix-Panabières, C. (2018). Autologous cell lines from circulating colon cancer cells captured from sequential liquid biopsies as model to study therapy-driven tumor changes. *Sci Rep*, 8(1), 15931. doi:10.1038/s41598-018-34365-z
- Sousa, B., Pereira, J., Marques, R., Grilo, L. F., Pereira, S. P., Sardão, V. A., . . . Paredes, J. (2020). P-cadherin induces anoikis-resistance of matrix-detached breast cancer cells by promoting pentose phosphate pathway and decreasing oxidative stress. *Biochim Biophys Acta Mol Basis Dis*, 1866(12), 165964. doi:10.1016/j.bbadis.2020.165964
- Sousa, B., Pereira, J., & Paredes, J. (2019). The Crosstalk Between Cell Adhesion and Cancer Metabolism. *Int J Mol Sci*, 20(8). doi:10.3390/ijms20081933
- Sousa, B., Ribeiro, A. S., Nobre, A. R., Lopes, N., Martins, D., Pinheiro, C., . . . Paredes, J. (2014). The basal epithelial marker P-cadherin associates with breast cancer cell populations harboring a glycolytic and acid-resistant phenotype. *BMC Cancer*, 14, 734. doi:10.1186/1471-2407-14-734
- Steinberg, G. R., & Kemp, B. E. (2009). AMPK in Health and Disease. *Physiol Rev*, 89(3), 1025-1078. doi:10.1152/physrev.00011.2008
- Stone, R. C., Pastar, I., Ojeh, N., Chen, V., Liu, S., Garzon, K. I., & Tomic-Canic, M. (2016). Epithelial-mesenchymal transition in tissue repair and fibrosis. *Cell Tissue Res*, 365(3), 495-506. doi:10.1007/s00441-016-2464-0

- Subbalakshmi, A. R., Kundnani, D., Biswas, K., Ghosh, A., Hanash, S. M., Tripathi, S. C., & Jolly, M. K. (2020). NFATc Acts as a Non-Canonical Phenotypic Stability Factor for a Hybrid Epithelial/Mesenchymal Phenotype. *Front Oncol*, 10, 553342. doi:10.3389/fonc.2020.553342
- Sun, Y. S., Zhao, Z., Yang, Z. N., Xu, F., Lu, H. J., Zhu, Z. Y., . . . Zhu, H. P. (2017). Risk Factors and Preventions of Breast Cancer. *Int J Biol Sci*, 13(11), 1387-1397. doi:10.7150/ijbs.21635
- Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*, 71(3), 209-249. doi:10.3322/caac.21660
- Suyama, K., Shapiro, I., Guttman, M., & Hazan, R. B. (2002). A signaling pathway leading to metastasis is controlled by N-cadherin and the FGF receptor. *Cancer Cell*, 2(4), 301-314. doi:10.1016/s1535-6108(02)00150-2
- Taherian, A., Li, X., Liu, Y., & Haas, T. A. (2011). Differences in integrin expression and signaling within human breast cancer cells. *BMC Cancer*, 11, 293. doi:10.1186/1471-2407-11-293
- Taniuchi, K., Nakagawa, H., Hosokawa, M., Nakamura, T., Eguchi, H., Ohigashi, H., . . . Nakamura, Y. (2005). Overexpressed P-cadherin/CDH3 promotes motility of pancreatic cancer cells by interacting with p120ctn and activating rho-family GTPases. *Cancer Res*, 65(8), 3092-3099. doi:10.1158/0008.5472.can-04-3646
- Theodoropoulos, P. A., Polioudaki, H., Agelaki, S., Kallergi, G., Saridaki, Z., Mavroudis, D., & Georgoulas, V. (2010). Circulating tumor cells with a putative stem cell phenotype in peripheral blood of patients with breast cancer. *Cancer Lett*, 288(1), 99-106. doi:10.1016/j.canlet.2009.06.027
- Thiery, J. P., Acloque, H., Huang, R. Y., & Nieto, M. A. (2009). Epithelial-mesenchymal transitions in development and disease. *Cell*, 139(5), 871-890. doi:10.1016/j.cell.2009.11.007
- Thorn, D. R., & Ladewig Hess, A. R. (2021). [Outpatient breast cancer treatment after the hospital: what's next? - Adjuvant medical therapies, management of side effects and common fears, planing and coordination of optimal follow-up care in view of current guidelines]. *Ther Umsch*, 78(3), 136-144. doi:10.1024/0040-5930/a001250
- Toiyama, Y., Yasuda, H., Saigusa, S., Tanaka, K., Inoue, Y., Goel, A., & Kusunoki, M. (2013). Increased expression of Slug and Vimentin as novel predictive biomarkers for lymph node metastasis and poor prognosis in colorectal cancer. *Carcinogenesis*, 34(11), 2548-2557. doi:10.1093/carcin/bgt282
- Tosoni, D., Pambianco, S., Ekalle Soppo, B., Zecchini, S., Bertalot, G., Pruneri, G., . . . Pece, S. (2017). Pre-clinical validation of a selective anti-cancer stem cell therapy for Numb-deficient human breast cancers. *EMBO Mol Med*, 9(5), 655-671. doi:10.15252/emmm.201606940
- Tran, M. N., Choi, W., Wszolek, M. F., Navai, N., Lee, I. L., Nitti, G., . . . McConkey, D. J. (2013). The p63 protein isoform ΔNp63α inhibits epithelial-mesenchymal transition in human bladder cancer cells: role of MIR-205. *J Biol Chem*, 288(5), 3275-3288. doi:10.1074/jbc.M112.408104
- Tucci, P., Agostini, M., Grespi, F., Markert, E. K., Terrinoni, A., Vousden, K. H., . . . Melino, G. (2012). Loss of p63 and its microRNA-205 target results in enhanced cell migration and metastasis in prostate cancer. *Proc Natl Acad Sci U S A*, 109(38), 15312-15317. doi:10.1073/pnas.1110977109
- Turashvili, G., McKinney, S. E., Goktepe, O., Leung, S. C., Huntsman, D. G., Gelmon, K. A., . . . Aparicio, S. A. (2011). P-cadherin expression as a prognostic biomarker in a 3992 case tissue microarray series of breast cancer. *Mod Pathol*, 24(1), 64-81. doi:10.1038/modpathol.2010.189
- Uemura, T., Shepherd, S., Ackerman, L., Jan, L. Y., & Jan, Y. N. (1989). numb, a gene required in determination of cell fate during sensory organ formation in Drosophila embryos. *Cell*, 58(2), 349-360. doi:10.1016/0092-8674(89)90849-0

- Urist, M. J., Di Como, C. J., Lu, M. L., Charytonowicz, E., Verbel, D., Crum, C. P., . . . Cordon-Cardo, C. (2002). Loss of p63 expression is associated with tumor progression in bladder cancer. *Am J Pathol*, 161(4), 1199-1206. doi:10.1016/s0002-9440(10)64396-9
- Valastyan, S., & Weinberg, R. A. (2011). Tumor metastasis: molecular insights and evolving paradigms. *Cell*, 147(2), 275-292. doi:10.1016/j.cell.2011.09.024
- Van Marck, V., Stove, C., Jacobs, K., Van den Eynden, G., & Bracke, M. (2011). P-cadherin in adhesion and invasion: opposite roles in colon and bladder carcinoma. *Int J Cancer*, 128(5), 1031-1044. doi:10.1002/ijc.25427
- van Roy, F. (2014). Beyond E-cadherin: roles of other cadherin superfamily members in cancer. *Nat Rev Cancer*, 14(2), 121-134. doi:10.1038/nrc3647
- Vandewalle, C., Comijn, J., De Craene, B., Vermassen, P., Bruyneel, E., Andersen, H., . . . Berx, G. (2005). SIP1/ZEB2 induces EMT by repressing genes of different epithelial cell-cell junctions. *Nucleic Acids Res*, 33(20), 6566-6578. doi:10.1093/nar/gki965
- Vandewalle, C., Van Roy, F., & Berx, G. (2009). The role of the ZEB family of transcription factors in development and disease. *Cell Mol Life Sci*, 66(5), 773-787. doi:10.1007/s00018-008-8465-8
- Vannier, C., Mock, K., Brabletz, T., & Driever, W. (2013). Zeb1 regulates E-cadherin and Epcam (epithelial cell adhesion molecule) expression to control cell behavior in early zebrafish development. *J Biol Chem*, 288(26), 18643-18659. doi:10.1074/jbc.M113.467787
- Varma, S., Cao, Y., Tagne, J. B., Lakshminarayanan, M., Li, J., Friedman, T. B., . . . Ramirez, M. I. (2012). The transcription factors Grainyhead-like 2 and NK2-homeobox 1 form a regulatory loop that coordinates lung epithelial cell morphogenesis and differentiation. *J Biol Chem*, 287(44), 37282-37295. doi:10.1074/jbc.M112.408401
- Vieira, A. F., & Paredes, J. (2015). P-cadherin and the journey to cancer metastasis. *Mol Cancer*, 14, 178. doi:10.1186/s12943-015-0448-4
- Vieira, A. F., Ribeiro, A. S., Dionísio, M. R., Sousa, B., Nobre, A. R., Albergaria, A., . . . Paredes, J. (2014). P-cadherin signals through the laminin receptor $\alpha 6\beta 4$ integrin to induce stem cell and invasive properties in basal-like breast cancer cells. *Oncotarget*, 5(3), 679-692. doi:10.18632/oncotarget.1459
- Vieira, A. F., Ricardo, S., Ablett, M. P., Dionísio, M. R., Mendes, N., Albergaria, A., . . . Paredes, J. (2012). P-cadherin is coexpressed with CD44 and CD49f and mediates stem cell properties in basal-like breast cancer. *Stem Cells*, 30(5), 854-864. doi:10.1002/stem.1075
- Voutilainen, S., Heikkilä, P., Sampo, M., Nevanlinna, H., Blomqvist, C., & Mattson, J. (2021). Expression of markers of stem cell characteristics, epithelial-mesenchymal transition, basal-like phenotype, proliferation, and androgen receptor in metaplastic breast cancer and their prognostic impact. *Acta Oncol*, 60(9), 1233-1239. doi:10.1080/0284186x.2021.1950927
- Wang, C., Jin, H., Wang, N., Fan, S., Wang, Y., Zhang, Y., . . . Qin, W. (2016). Gas6/Axl Axis Contributes to Chemoresistance and Metastasis in Breast Cancer through Akt/GSK-3 β / β -catenin Signaling. *Theranostics*, 6(8), 1205-1219. doi:10.7150/thno.15083
- Wang, H., Chen, H., Jiang, Z., Lin, Y., Wang, X., Xiang, J., & Peng, J. (2019). Integrin subunit alpha V promotes growth, migration, and invasion of gastric cancer cells. *Pathol Res Pract*, 215(9), 152531. doi:10.1016/j.prp.2019.152531
- Wang, H., Stoecklein, N. H., Lin, P. P., & Gires, O. (2017). Circulating and disseminated tumor cells: diagnostic tools and therapeutic targets in motion. *Oncotarget*, 8(1), 1884-1912. doi:10.18632/oncotarget.12242
- Wang, P., Lin, S. L., Zhang, L. H., Li, Z., Liu, Q., Gao, J. X., . . . Huang, Y. R. (2014). The prognostic value of P-cadherin in non-muscle-invasive bladder cancer. *Eur J Surg Oncol*, 40(3), 255-259. doi:10.1016/j.ejso.2013.12.018

- Wang, X., Pan, X., & Song, J. (2010). AMP-activated protein kinase is required for induction of apoptosis and epithelial-to-mesenchymal transition. *Cell Signal*, 22(11), 1790-1797. doi:10.1016/j.cellsig.2010.07.008
- Wang, X. J., Sun, Z., Villeneuve, N. F., Zhang, S., Zhao, F., Li, Y., . . . Zhang, D. D. (2008). Nrf2 enhances resistance of cancer cells to chemotherapeutic drugs, the dark side of Nrf2. *Carcinogenesis*, 29(6), 1235-1243. doi:10.1093/carcin/bgn095
- Wang, Y., Shi, J., Chai, K., Ying, X., & Zhou, B. P. (2013). The Role of Snail in EMT and Tumorigenesis. *Curr Cancer Drug Targets*, 13(9), 963-972. doi:10.2174/15680096113136660102
- Wang, Z., Sandiford, S., Wu, C., & Li, S. S. (2009). Numb regulates cell-cell adhesion and polarity in response to tyrosine kinase signalling. *Embo j*, 28(16), 2360-2373. doi:10.1038/emboj.2009.190
- Wang, Z. H., Li, Z., Hu, M., Yang, Q. J., Yan, S., Wu, R. S., . . . Guo, M. (2017). Ovol2 gene inhibits the Epithelial-to-Mesenchymal Transition in lung adenocarcinoma by transcriptionally repressing Twist1. *Gene*, 600, 1-8. doi:10.1016/j.gene.2016.11.034
- Watanabe, K., Villarreal-Ponce, A., Sun, P., Salmans, M. L., Fallahi, M., Andersen, B., & Dai, X. (2014). Mammary morphogenesis and regeneration require the inhibition of EMT at terminal end buds by Ovol2 transcriptional repressor. *Dev Cell*, 29(1), 59-74. doi:10.1016/j.devcel.2014.03.006
- Watkins, E. J. (2019). Overview of breast cancer. *Jaapa*, 32(10), 13-17. doi:10.1097/01.JAA.0000580524.95733.3d
- Welch, D. R., & Hurst, D. R. (2019). Defining the Hallmarks of Metastasis. *Cancer Res*, 79(12), 3011-3027. doi:10.1158/0008-5472.Can-19-0458
- Werner, S., Frey, S., Riethdorf, S., Schulze, C., Alawi, M., Kling, L., . . . Assmann, V. (2013). Dual roles of the transcription factor grainyhead-like 2 (GRHL2) in breast cancer. *J Biol Chem*, 288(32), 22993-23008. doi:10.1074/jbc.M113.456293
- Werner, S., Stenzl, A., Pantel, K., & Todenhöfer, T. (2017). Expression of Epithelial Mesenchymal Transition and Cancer Stem Cell Markers in Circulating Tumor Cells. *Adv Exp Med Biol*, 994, 205-228. doi:10.1007/978-3-319-55947-6_11
- Wheelock, M. J., Shintani, Y., Maeda, M., Fukumoto, Y., & Johnson, K. R. (2008). Cadherin switching. *J Cell Sci*, 121(Pt 6), 727-735. doi:10.1242/jcs.000455
- WHO. (2021, 3 March 2021). Cancer. Retrieved from <https://www.who.int/news-room/fact-sheets/detail/cancer>
- Willows, R., Sanders, M. J., Xiao, B., Patel, B. R., Martin, S. R., Read, J., . . . Carling, D. (2017). Phosphorylation of AMPK by upstream kinases is required for activity in mammalian cells. *Biochem J*, 474(17), 3059-3073. doi:10.1042/bcj20170458
- Wolf, G. T., Carey, T. E., Schmaltz, S. P., McClatchey, K. D., Poore, J., Glaser, L., . . . Hsu, S. (1990). Altered antigen expression predicts outcome in squamous cell carcinoma of the head and neck. *J Natl Cancer Inst*, 82(19), 1566-1572. doi:10.1093/jnci/82.19.1566
- Wong, S. H. M., Fang, C. M., Chuah, L. H., Leong, C. O., & Ngai, S. C. (2018). E-cadherin: Its dysregulation in carcinogenesis and clinical implications. *Crit Rev Oncol Hematol*, 121, 11-22. doi:10.1016/j.critrevonc.2017.11.010
- Woo, D., & Yu, M. (2018). Circulating tumor cells as "liquid biopsies" to understand cancer metastasis. *Transl Res*, 201, 128-135. doi:10.1016/j.trsl.2018.07.003
- Wood, B., & Leong, A. (2003). The biology and diagnostic applications of cadherins in neoplasia: a review. *Pathology*, 35(2), 101-105.
- Wu, G., Nomoto, S., Hoque, M. O., Dracheva, T., Osada, M., Lee, C. C., . . . Trink, B. (2003). DeltaNp63alpha and Tap63alpha regulate transcription of genes with distinct biological functions in cancer and development. *Cancer Res*, 63(10), 2351-2357.
- Wu, H. T., Zhong, H. T., Li, G. W., Shen, J. X., Ye, Q. Q., Zhang, M. L., & Liu, J. (2020). Oncogenic functions of the EMT-related transcription factor ZEB1 in breast cancer. *J Transl Med*, 18(1), 51. doi:10.1186/s12967-020-02240-z

- Wu, S., Liu, S., Liu, Z., Huang, J., Pu, X., Li, J., . . . Xu, J. (2015). Classification of circulating tumor cells by epithelial-mesenchymal transition markers. *PLoS One*, 10(4), e0123976. doi:10.1371/journal.pone.0123976
- Wu, S., Lu, H., & Bai, Y. (2019). Nrf2 in cancers: A double-edged sword. *Cancer Med*, 8(5), 2252-2267. doi:10.1002/cam4.2101
- Xia, M., Hu, M., Wang, J., Xu, Y., Chen, X., Ma, Y., & Su, L. (2010). Identification of the role of Smad interacting protein 1 (SIP1) in glioma. *J Neurooncol*, 97(2), 225-232. doi:10.1007/s11060-009-0015-1
- Xiang, X., Deng, Z., Zhuang, X., Ju, S., Mu, J., Jiang, H., . . . Zhang, H. G. (2012). Grhl2 determines the epithelial phenotype of breast cancers and promotes tumor progression. *PLoS One*, 7(12), e50781. doi:10.1371/journal.pone.0050781
- Xiang, X., Saha, A. K., Wen, R., Ruderman, N. B., & Luo, Z. (2004). AMP-activated protein kinase activators can inhibit the growth of prostate cancer cells by multiple mechanisms. *Biochem Biophys Res Commun*, 321(1), 161-167. doi:10.1016/j.bbrc.2004.06.133
- Xing, X., Tang, Y. B., Yuan, G., Wang, Y., Wang, J., Yang, Y., & Chen, M. (2013). The prognostic value of E-cadherin in gastric cancer: a meta-analysis. *Int J Cancer*, 132(11), 2589-2596. doi:10.1002/ijc.27947
- Xu, H., Tian, Y., Yuan, X., Liu, Y., Wu, H., Liu, Q., . . . Wu, K. (2016). Enrichment of CD44 in basal-type breast cancer correlates with EMT, cancer stem cell gene profile, and prognosis. *Onco Targets Ther*, 9, 431-444. doi:10.2147/ott.S97192
- Xu, Y., Qin, L., Sun, T., Wu, H., He, T., Yang, Z., . . . Xu, J. (2017). Twist1 promotes breast cancer invasion and metastasis by silencing Foxa1 expression. *Oncogene*, 36(8), 1157-1166. doi:10.1038/onc.2016.286
- Yang, J., Mani, S. A., Donaher, J. L., Ramaswamy, S., Itzykson, R. A., Come, C., . . . Weinberg, R. A. (2004). Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell*, 117(7), 927-939. doi:10.1016/j.cell.2004.06.006
- Yang, Y., Guan, Q., Guo, L., & Han, C. (2018). The prognostic correlation between CD105 expression level in tumor tissue and peripheral blood and sunitinib administration in advanced hepatocellular carcinoma. *Cancer Biol Ther*, 19(11), 1006-1014. doi:10.1080/15384047.2018.1470731
- Yilmaz, M., & Christofori, G. (2009). EMT, the cytoskeleton, and cancer cell invasion. *Cancer Metastasis Rev*, 28(1-2), 15-33. doi:10.1007/s10555-008-9169-0
- Yin, L., Duan, J. J., Bian, X. W., & Yu, S. C. (2020). Triple-negative breast cancer molecular subtyping and treatment progress. *Breast Cancer Res*, 22(1), 61. doi:10.1186/s13058-020-01296-5
- Yun, J. H., Diaz, R., & Orman, A. G. (2018). Breast Reconstruction and Radiation Therapy. *Cancer Control*, 25(1), 1073274818795489. doi:10.1177/1073274818795489
- Zhang, C. C., Yan, Z., Zhang, Q., Kuszpit, K., Zasadny, K., Qiu, M., . . . VanArsdale, T. (2010). PF-03732010: a fully human monoclonal antibody against P-cadherin with antitumor and antimetastatic activity. *Clin Cancer Res*, 16(21), 5177-5188. doi:10.1158/1078-0432.ccr-10-1343
- Zhang, D., Bi, J., Liang, Q., Wang, S., Zhang, L., Han, F., . . . Ding, Y. (2020). VCAM1 Promotes Tumor Cell Invasion and Metastasis by Inducing EMT and Transendothelial Migration in Colorectal Cancer. *Front Oncol*, 10, 1066. doi:10.3389/fonc.2020.01066
- Zhang, H., Singh, R. R., Talukder, A. H., & Kumar, R. (2006). Metastatic tumor antigen 3 is a direct corepressor of the Wnt4 pathway. *Genes Dev*, 20(21), 2943-2948. doi:10.1101/gad.1461706
- Zhang, J., Shao, X., Sun, H., Liu, K., Ding, Z., Chen, J., . . . Li, H. (2016). NUMB negatively regulates the epithelial-mesenchymal transition of triple-negative breast cancer by antagonizing Notch signaling. *Oncotarget*, 7(38), 61036-61053. doi:10.18632/oncotarget.11062
- Zhang, L., & Yu, D. (2019). Exosomes in cancer development, metastasis, and immunity. *Biochim Biophys Acta Rev Cancer*, 1871(2), 455-468. doi:10.1016/j.bbcan.2019.04.004

- Zhang, X., Zhang, Z., Zhang, Q., Zhang, Q., Sun, P., Xiang, R., . . . Yang, S. (2018). ZEB1 confers chemotherapeutic resistance to breast cancer by activating ATM. *Cell Death Dis*, 9(2), 57. doi:10.1038/s41419-017-0087-3
- Zhao, X., Sun, B., Sun, D., Liu, T., Che, N., Gu, Q., . . . Li, J. (2015). Slug promotes hepatocellular cancer cell progression by increasing sox2 and nanog expression. *Oncol Rep*, 33(1), 149-156. doi:10.3892/or.2014.3562
- Zhu, C., Kong, Z., Wang, B., Cheng, W., Wu, A., & Meng, X. (2019). ITGB3/CD61: a hub modulator and target in the tumor microenvironment. *Am J Transl Res*, 11(12), 7195-7208.
- Zhuo, X., Chang, A., Huang, C., Yang, L., Xiang, Z., & Zhou, Y. (2015). Expression and clinical significance of microvessel density and its association with TWIST in nasopharyngeal carcinoma. *Int J Clin Exp Med*, 8(1), 1265-1270.
- Zou, M., Zhu, W., Wang, L., Shi, L., Gao, R., Ou, Y., . . . Hu, G. (2016). AEG-1/MTDH-activated autophagy enhances human malignant glioma susceptibility to TGF- β 1-triggered epithelial-mesenchymal transition. *Oncotarget*, 7(11), 13122-13138. doi:10.18632/oncotarget.7536

7. Supplementary Data

Gene	CDH3	CDH1	CDH2	EPCAM	GRHL2	OCN	TJP1	CTNBN1	VIM	CD44	ITGB3	SNAI2	SNAI1	ZEB2	ZEB1	TP63	ITGB4	VCAM1	ITGAV	TWIST1	NFE2L2	OVOL2	NUMB	PRKAA2	SMAD3
AU565_BREAST	-5.515	-4.0403	-4.0198	6.4428	3.2638	3.6197	3.8374	5.1598	0.1882	1.5926	-4.7446	-4.7287	0.5432	-6.685	-5.163	-3.601	3.161	-4.9301	4.2739	-6.0214	3.5738	2.8073	3.9665	-4.6374	1.7782
HS343T_BREAST	-4.8619	-2.2643	6.4446	-2.262	-5.7505	-1.9277	3.4277	5.3989	10.709	7.0233	2.472	4.8306	2.3935	1.7227	4.1818	-5.7758	-3.8447	-2.9775	5.5897	3.2103	4.151	-6.4261	3.5986	-2.7792	3.5962
HS281T_BREAST	-4.8531	-2.2377	6.2209	-1.783	-6.6505	-2.7295	3.5408	5.4891	10.782	6.2832	3.5146	5.0701	0.6206	2.5557	3.892	-5.5571	-2.9538	-1.0849	5.3961	3.1147	4.0525	-7.1334	3.4552	-1.1918	2.7696
HMC18_BREAST	-4.6294	-3.7237	3.9037	-2.3797	-6.1257	1.5176	3.5333	5.7539	8.8638	2.6665	-0.9927	4.0706	0.0639	0.6062	3.232	-4.5135	-4.1004	-3.4782	3.7488	3.5017	5.3292	-3.8013	2.9618	1.0853	1.4277
HS274T_BREAST	-4.3636	-0.9771	4.8136	-2.007	-13	-0.9115	4.5238	5.6088	9.9356	6.1703	1.7287	5.4596	0.0341	3.539	3.4362	-7.1844	-3.1241	-6.0642	6.2249	1.7446	4.9103	-5.5128	3.2307	-1.023	4.6515
HS739T_BREAST	-3.7091	-1.9354	7.0825	-1.4196	-5.9278	-2.7313	3.3579	5.5833	9.8558	5.8311	1.3251	5.1701	1.5444	1.9586	3.4282	-6.2751	-2.8701	2.1507	5.5841	3.1754	4.2338	-13	3.3494	-1.8787	3.2548
MDAMB436_BREAS	-3.679	-3.4232	4.6876	0.0852	-5.6931	-0.1667	3.8379	5.6989	7.8815	7.1926	-2.4936	1.8894	2.0329	2.9595	1.7339	-2.196	2.7428	-1.4488	5.1962	3.7609	4.7037	-5.5062	3.2619	2.0648	3.7647
HS606T_BREAST	-3.4893	-2.5684	6.8298	-2.7536	-6.1065	-4.47	3.6477	5.5653	10.493	6.7553	1.2557	4.6391	2.9198	1.3867	4.2033	-6.6058	-3.2497	2.0767	5.3984	3.0424	4.5102	-7.1041	3.5526	-1.3106	3.9508
SKBR3_BREAST	-3.4086	-2.6435	-3.2271	6.2071	3.0446	3.238	3.0039	5.1495	0.2015	-0.361	-4.1038	-4.5474	0.7222	-5.5512	-6.0077	-4.6506	3.0269	-4.5748	3.4619	-6.9657	3.6085	2.4371	3.6267	-4.9571	2.0655
HS578T_BREAST	-3.2924	-2.3538	7.1532	-2.7796	-6.6955	-1.4577	3.3455	5.2783	10.176	6.2315	3.6157	6.2731	0.8474	2.4563	4.0897	-2.9838	-3.7898	-2.42	5.9551	3.3383	4.1193	-7.9561	2.9787	-1.044	3.8301
MDAMB231_BREAS	-3.2239	-1.4062	-1.0744	3.334	-3.2776	0.7173	3.5425	6.2832	8.9811	7.2671	0.4672	3.9509	0.973	-0.1531	3.076	-2.8609	5.6756	-1.6476	4.1517	-4.8161	4.5788	-13	3.4897	0.856	4.9625
HS742T_BREAST	-3.1799	-2.782	6.8262	-2.6654	-7.6534	-4.0418	2.8605	5.1726	9.5377	6.8688	2.7737	2.5881	1.9625	1.0219	3.6296	-6.0007	-3.7477	-0.0391	5.9893	1.1771	3.4505	-13	3.1937	-0.3378	3.0335
MDAMB157_BREAS	-0.9659	-2.5624	3.4098	0.2942	-4.2477	0.5031	2.2633	5.4568	9.7518	4.6617	2.1934	6.4619	4.0403	2.2101	1.7226	-1.1483	0.5097	2.1876	5.7409	5.5011	4.6314	-3.4181	3.5573	-7.6988	4.1734
HCC2218_BREAST	-0.8276	-2.7859	-4.1836	6.1009	3.9635	3.7225	2.9001	4.6436	-0.5538	1.0323	-0.7928	-4.429	-0.0515	-8.0958	-6.3182	-6.7991	6.4443	-4.3569	3.2674	-6.0697	2.9867	1.9142	2.523	-0.7747	2.3102
CAL120_BREAST	-0.7477	3.058	4.2727	1.6395	-0.7677	1.9574	3.5266	4.626	9.0088	5.4102	-4.0768	2.7427	-1.3832	1.4242	1.5227	-7.083	5.9654	-3.4268	4.0528	2.7113	4.2946	-4.0329	2.8207	2.3323	2.5702
HCC1500_BREAST	-0.3199	6.888	1.7113	6.6185	5.4904	4.021	2.0417	5.575	-0.4215	3.6597	-3.2524	-3.1365	0.7549	-6.2184	-4.1715	-3.5065	-0.8273	-4.1384	3.1779	1.7086	3.9784	2.5882	3.0038	2.3452	2.3621
MDAMB134VI_BRE	0.1358	2.6172	2.6924	6.6119	5.0446	2.2575	1.5239	4.338	1.8782	5.2854	-4.7327	-6.4982	-0.1667	-7.165	-3.3735	-3.6014	-2.6203	-5.6187	2.2667	1.4466	3.8633	2.9417	3.2828	1.0317	1.5316
MDAMB453_BREAS	1.4603	3.2274	-1.8159	6.8204	4.7727	2.6117	3.5355	5.7166	-0.7076	-2.957	-4.3182	-4.9842	-2.8845	-6.6509	-4.7075	-0.8144	3.3895	-7.0115	3.663	-13	4.5948	3.0751	3.7963	1.4411	1.8008
BT474_BREAST	1.959	7.3197	0.2289	6.4202	4.9145	2.897	4.0439	5.3662	2.9945	1.3572	-3.54	-3.543	-1.1644	-6.8728	-5.6663	-0.5495	2.2921	-6.6484	3.108	2.5482	3.9004	2.8317	3.147	0.0014	1.0789
CAMA1_BREAST	2.4375	4.4697	-2.8588	6.4367	5.2921	3.0183	3.2633	5.9678	0.2355	-0.3497	-3.546	-2.4046	0.2545	-6.5689	-3.9389	-3.6696	2.3044	-5.1101	3.1116	1.9845	3.9162	3.1521	3.4061	-6.2164	1.2588
HCC1395_BREAST	2.4507	-1.312	5.7128	-0.2575	-4.0106	-0.1728	3.387	6.7381	8.2771	5.1672	2.8183	4.692	3.4695	0.0412	2.9424	-0.7233	2.0748	-3.2376	5.8485	2.2722	5.4643	0.989	3.1651	-4.7392	5.871
MDAMB415_BREAS	2.7332	6.8143	-2.0218	6.6575	4.0446	2.6006	3.8858	4.6789	0.9077	4.4577	-3.1201	-1.9289	-0.775	-6.5715	-4.5494	2.41	3.8429	-4.7946	3.8334	-7.6449	4.2758	2.1616	3.429	0.5763	2.4531
CAL51_BREAST	2.7739	3.8806	2.1693	7.5649	-4.2633	1.6865	4.4102	6.3097	4.9963	4.8951	4.2015	-3.1634	0.1882	-4.1113	3.0865	-6.726	3.6017	-5.0209	6.4897	4.203	4.6203	-2.595	4.0906	-3.2434	3.1613
HCC1419_BREAST	2.8509	6.2039	-3.7716	6.4922	5.6418	2.4947	1.9411	5.4544	-0.2631	0.173	-4.9835	-6.4271	1.7031	-7.8714	-5.3348	-5.6678	4.2341	-6.1325	4.2557	3.0698	4.1463	0.9203	2.7693	1.1922	1.1526
UACC812_BREAST	3.2402	6.7043	-2.0205	6.333	4.7104	3.9194	2.4712	4.7686	1.5053	3.2827	-2.2395	-1.8086	0.9236	-4.9713	-4.4652	-0.8718	3.8997	-4.8624	2.7218	-2.2939	3.0846	1.4047	3.5065	0.5731	2.5151
HCC1187_BREAST	3.3711	4.8187	4.0206	4.7873	5.2297	2.3688	4.3782	6.4286	4.3098	1.691	-4.1531	-1.8962	2.9328	-2.2907	-3.9194	-3.5986	1.1477	5.8802	5.766	-1.5819	4.4009	0.2989	3.9215	-3.2663	1.5742
HCC1569_BREAST	3.4262	5.109	-1.4465	6.6146	3.2268	0.7177	2.5095	8.8259	8.0042	7.2657	-0.8768	7.2604	0.7369	-6.5017	-3.8098	-3.8126	-2.0451	-3.4997	4.7499	-4.1537	3.8405	1.9942	2.6808	-0.6652	2.8474
HCC1428_BREAST	3.7022	7.455	-3.924	6.1037	5.2847	2.0511	3.3011	4.8372	-0.458	4.703	-3.4458	-4.6549	0.9874	-7.3217	-5.0586	-2.0882	2.7484	-5.2493	3.6247	-3.9032	3.864	2.618	3.0829	0.7296	2.1538
ZR7530_BREAST	3.7037	1.5568	-3.7057	6.2722	5.1287	2.7097	3.6999	5.4258	0.5457	3.0227	-3.1825	-4.4145	-1.9137	-6.1484	-5.4862	-5.1887	3.5191	-4.8569	2.7782	-2.7531	3.893	2.6592	3.2573	3.0057	3.4963
MCF7_BREAST	3.7517	7.7217	-4.2814	7.5063	4.81	3.2884	3.1045	5.5864	-0.7169	3.0964	-3.4577	-3.3707	0.1016	-6.738	-4.3615	-1.7451	4.8674	-8.5136	4.0488	-0.8601	3.6848	0.049	3.3307	-4.9761	3.0328
BT483_BREAST	3.928	7.8594	-1.5772	6.4925	5.1877	3.6946	2.9473	5.7423	0.274	1.4932	-3.3093	-2.9593	0.2364	-5.803	-5.442	-4.0525	3.4767	-6.5172	3.1025	-2.2836	3.841	2.7438	2.4982	0.567	1.7321
T47D_BREAST	4.0403	7.1027	-4.6154	5.9563	5.6839	2.7983	3.0694	6.1806	-0.2538	1.864	-3.7466	-4.7206	-1.2839	-6.8569	-5.5029	-3.0438	5.0311	-7.333	3.6138	-3.0234	3.1613	2.081	4.2531	1.0596	0.8352
MDAMB468_BREAS	4.1826	4.8955	0.679	7.3918	4.5652	2.7957	3.4856	6.0515	2.76	7.6851	-2.1093	-3.0185	0.2642	-5.1708	-3.0032	-1.4421	4.9024	-1.5865	5.284	-2.4368	4.993	2.3634	3.3752	2.1738	3.1613
CAL148_BREAST	4.1962	5.7707	-4.4514	7.2007	4.694	3.7303	3.1779	6.3797	0.4245	-1.4506	-4.3855	-4.1186	-0.7538	-8.1479	-5.8228	-3.7014	0.7116	-4.2074	3.1675	0.2401	3.978	3.8113	2.9433	0.0856	-0.5128
MDAMB175VII_BRE	4.2455	7.6449	-1.7768	5.6855	4.5853	3.4734	3.67	5.8217	0.2624	-0.2057	-3.4158	-2.1869	-3.6153	-7.0667	-4.8342	-1.8147	6.0222	-5.1318	4.2099	0.4366	4.3773	1.8252	3.8308	-1.3313	4.6249
ZR751_BREAST	4.3153	6.7486	-1.4339	6.9532	4.5799	3.4601	2.7421	5.228	1.8034	2.3649	-2.0285	-3.2311	-0.6619	-4.7403	-3.8557	-2.5634	3.1855	-3.3739	3.977	1.3226	4.062	2.5719	3.4228	-0.0648	2.3135
HCC1599_BREAST	4.5366	5.0034	-3.0023	8.0357	4.9084	3.595	2.2578	6.851	4.4125	4.6557	-2.6994	-3.1429	0.037	-7.5259	-3.3194	-5.4218	1.8153	-5.524	3.2142	0.2281	5.5519	2.4111	2.7507	1.8278	3.4487
DU4475_BREAST	4.8043	4.5209	-6.2784	7.2931	1.5224	2.6217	3.1241	7.9292	-0.6472	-2.3808	0.5834	-2.0145	-0.2374	-2.4115	0.414	-6.724	7.0407	-7.4111	2.6935	-5.802	4.1106	1.7605	2.5753	-8.4179	-0.1313
KPL1_BREAST	4.8393	7.374	-3.232	7.3752	4.7088	3.2967	3.7084	5.7502	0.4821	-0.1742	-3.6453	-3.3727	0.7204	-6.3931	-5.0346	0.109	5.7305	-8.3387	3.7632	0.6841	3.6628	0.4981	3.7426	0.0165	4.2952
EFM19_BREAST	4.8778	6.5521	-3.833	6.4988	5.1158	2.5798	3.5616	8.8003	1.0709	2.0357	-4.4817	-5.3403	2.0013	-6.0071	-6.3856	0.5496	2.8403	-6.3677	2.9633	-7.7586	3.8453	1.1055	6.3397	-0.5061	2.3784
BT549_BREAST	5.1392	-1.6439	5.7128	-1.8203	-5.0801	1.0086	2.7943	5.7566	9.0449	7.9491	-0.7115	4.9833	0.684	1.1362	3.3198	-2.405	-2.0776	-2.4998	5.061	5.1283	4.1744	-13	2.7769	-3.1352	3.1912
MDAMB361_BREAS	5.2617	7.9422	3.1555	7.3829	4.8253	2.1023	2.7789	5.4004	7.1862	5.3447	2.0425	-2.2178	1.4177	-2.486	-0.0714	-0.756	5.6164	-5.5347	3.7554	0.231	4.3575	2.1494	3.0418	0.3654	3.2521
UACC																									

Gene	Gene cluster	CDH3	CDH1	CDH2	EPCAM	GRHL2	OCLN	TJP1	CTNNB1	VIM	CD44	ITGB3	SNAI2	SNAI1	ZEB2	ZEB1	TP63	ITGB4	VCAM1	ITGAV	TWIST1	NFE2L2	OVOL2	NUMB	PRKAA2	SMAD3
HCC1187_BREAST	Basal A	3,3711	4,8187	4,0206	4,7873	5,2297	2,3688	4,3782	6,4286	4,3098	1,691	-4,1531	-1,8962	2,9328	-2,2907	-3,9194	-3,5986	1,1477	5,8802	5,766	-1,5819	4,4009	0,2989	3,9215	-3,2663	1,5742
HCC1569_BREAST	Basal A	3,4262	5,109	-1,4465	6,6146	3,2268	0,7177	2,5095	5,8259	8,0042	7,2657	-0,8768	7,2604	0,7369	-6,5017	-3,8098	-3,8126	-2,0451	-3,4997	4,7499	-4,1537	3,8405	1,9942	2,6808	-0,6652	2,8474
MDAMB468_BREAST	Basal A	4,1826	4,8955	0,679	7,3918	4,5652	2,7857	3,4856	6,0515	2,76	7,6851	-2,1093	-3,0185	0,2642	-5,1708	-3,0032	-1,4421	4,9024	-1,5865	5,284	-2,4368	4,993	2,3634	3,3752	2,1738	3,1613
HCC2157_BREAST	Basal A	5,4187	5,5391	-3,3625	6,5396	4,4723	3,3142	2,4083	4,0447	7,582	5,6433	-6,0748	-4,5184	2,1196	-6,3778	-3,9083	-4,7118	2,2211	-4,5458	2,4553	-6,9367	3,2023	2,7538	2,6074	-0,7092	1,3465
HCC1143_BREAST	Basal A	6,4975	6,4674	4,6802	7,145	5,0512	4,127	4,3229	7,772	8,0981	6,9439	2,8583	2,8254	2,3252	-4,3632	-0,2686	-3,4546	7,2204	-13	6,0406	2,4433	4,231	2,921	3,7497	-0,1188	5,7519
HCC1937_BREAST	Basal A	6,6108	6,8839	-2,9563	7,1143	4,4142	3,1846	3,5861	4,9825	0,8292	6,9666	-3,5749	1,7995	0,6662	-6,5248	-4,0055	-0,4398	4,7495	-4,0889	4,9769	-5,9392	3,9414	1,8375	3,8705	-5,6166	3,6653
HCC1954_BREAST	Basal A	6,8948	7,3268	-1,1445	6,5641	5,3989	3,4542	3,8228	6,724	3,5114	6,0842	0,8349	2,9809	0,3182	-4,4509	-3,4701	4,5599	5,8163	-6,0845	5,0549	-3,5685	4,8719	1,3434	3,8146	0,5501	3,1691
BT20_BREAST	Basal A	7,3804	7,5055	-3,6528	6,6355	5,0127	2,6552	4,228	5,7379	0,9585	6,0676	-3,7022	2,3512	1,3098	-6,3056	-2,8096	-0,5196	4,8545	-7,8361	5,6244	-5,9051	5,4061	2,5826	3,865	0,8587	4,4013
HCC70_BREAST	Basal A	7,3929	7,3357	-3,5275	5,9545	4,8394	3,6376	3,9235	5,4434	3,0059	5,5024	-4,031	2,8168	2,1119	-4,1578	-3,3586	-0,5482	4,2909	-5,569	5,5177	-5,7673	5,2075	2,5738	4,1317	2,0507	4,5042
MDAMB436_BREAST	Basal B	-3,679	-3,4232	4,6876	0,0852	-5,6931	-0,1667	3,8379	5,6989	7,8815	7,1926	-2,4936	1,8894	2,0329	2,9595	1,7339	-2,196	2,7428	-1,4488	5,1962	3,7609	4,7037	-5,5062	3,2619	2,0648	3,7647
HS578T_BREAST	Basal B	-3,2924	-2,3538	7,1532	-2,7796	-6,6955	-1,4577	3,3455	5,2783	10,176	6,2315	3,6157	6,2731	0,8474	2,4563	4,0897	-2,9838	-3,7898	-2,42	5,9551	3,3383	4,1193	-7,9561	2,9787	-1,044	3,8301
MDAMB231_BREAST	Basal B	-3,2239	-1,4062	-1,0744	3,334	-3,2776	0,7173	3,5425	6,2832	8,9811	7,2671	0,4672	3,9509	0,973	-0,1531	3,076	-2,8609	5,6756	-1,6476	4,1517	-4,8161	4,5788	-13	3,4897	0,856	4,9625
MDAMB157_BREAST	Basal B	-0,9659	-2,5624	3,4098	0,2942	-4,2477	0,5031	2,2633	5,4568	9,7518	4,6617	2,1934	6,4619	4,0403	2,2101	1,7226	-1,1483	0,5097	2,1876	5,7409	5,5011	4,6314	-3,4181	3,5573	-7,6988	4,1734
HCC1500_BREAST	Basal B	-0,3199	6,888	1,7113	6,6185	5,4904	4,021	2,0417	5,575	-0,4215	3,6597	-3,2524	-3,1365	0,7549	-6,2184	-4,1715	-3,5065	-0,8273	-4,1384	3,1779	1,7086	3,9784	2,5882	3,0038	2,3452	2,3621
BT549_BREAST	Basal B	5,1392	-1,6439	5,7128	-1,8203	-5,0801	1,0086	2,7943	5,7566	9,0449	7,9491	-0,7115	4,9833	0,684	1,1362	3,3198	-2,405	-2,0776	-2,4998	5,061	5,1283	4,1744	-13	2,7769	-3,1352	3,1912
HCC38_BREAST	Basal B	6,2461	5,7116	2,7855	7,6097	4,635	2,7074	4,5194	7,3952	5,8208	6,0446	4,4787	0,5528	2,5174	-3,6222	-1,7134	-0,8664	4,4279	-4,9068	5,2331	-0,0973	4,0351	1,9455	3,938	-0,8862	4,4638
AU565_BREAST	Luminal	-5,515	-4,0403	-4,0198	6,4428	3,2638	3,6197	3,8374	5,1598	0,1882	1,5926	-4,7446	-4,7287	0,5432	-6,685	-5,163	-3,601	3,161	-4,9301	4,2739	-6,0214	3,5738	2,8073	3,9665	-4,6374	1,7782
SKBR3_BREAST	Luminal	-3,4086	-2,6435	-3,2271	6,2071	3,0446	3,238	3,0039	5,1495	0,2015	-0,361	-4,1038	-4,5474	0,7222	-5,5512	-6,0077	-4,6506	3,0269	-4,5748	3,4619	-6,9657	3,6085	2,4371	3,6267	-4,9571	2,0655
MDAMB134VI_BREAST	Luminal	0,1358	2,6172	2,6924	6,6119	5,0446	2,2575	1,5239	4,338	1,8782	5,2854	-4,7327	-6,4982	-0,1667	-7,165	-3,3735	-3,6014	-2,6203	-5,6187	2,2667	1,4466	3,8633	2,9417	2,3828	1,0317	1,5316
MDAMB453_BREAST	Luminal	1,4603	3,2274	-1,8159	6,8204	4,7727	2,6117	3,5355	5,7166	-0,7076	-2,957	-4,3182	-4,9842	-2,8845	-6,6509	-4,7075	-0,8144	3,3895	-7,0115	3,663	-13	4,5948	3,0751	3,7963	1,4411	1,8008
BT474_BREAST	Luminal	1,959	7,3197	0,2289	6,4202	4,9145	2,897	4,0439	5,3662	2,9945	1,3572	-3,54	-3,543	-1,1644	-6,8728	-5,6663	-0,5495	2,2921	-6,6484	3,108	2,5482	3,9004	2,8317	3,147	0,0014	1,0789
CAMA1_BREAST	Luminal	2,4375	4,4697	-2,8588	6,4367	5,2921	3,0183	3,2633	5,9678	0,2355	-0,3497	-3,546	-2,4046	0,2545	-6,5689	-3,9389	-3,6696	2,3044	-5,1101	3,1116	1,9845	3,9162	3,1521	3,4061	-6,2164	1,2588
MDAMB415_BREAST	Luminal	2,7332	6,8143	-2,0218	6,6575	4,0446	2,6006	3,8858	4,6789	0,9077	4,4577	-3,1201	-1,9289	-0,775	-6,5715	-4,5494	2,41	3,8429	-4,7946	3,8334	-7,6449	4,2758	2,1616	3,429	0,5763	2,4531
UACC812_BREAST	Luminal	3,2402	6,7043	-2,0205	6,333	4,7104	3,9194	2,4712	4,7686	1,5053	3,2827	-2,2395	-1,8086	0,9236	-4,9713	-4,4652	-0,8718	3,8997	-4,8624	2,7218	-2,2939	3,0846	1,4047	3,5065	0,5731	2,5151
HCC1428_BREAST	Luminal	3,7022	7,455	-3,924	6,1037	5,2847	2,0511	3,3011	4,8372	-0,458	4,703	-3,4458	-6,4549	0,9874	-7,3217	-5,0586	-2,0882	2,7484	-5,2493	3,6247	-3,9032	3,864	2,618	3,0829	0,7296	2,1538
ZR7530_BREAST	Luminal	3,7037	1,5568	-3,7057	6,2722	5,1287	2,7097	3,6999	5,4258	0,5457	3,0227	-3,1825	-4,4145	-1,9137	-6,1484	-5,4862	-5,1887	3,5191	-4,8569	2,7782	-2,7531	3,893	2,6592	3,2573	3,0057	3,4963
MCF7_BREAST	Luminal	3,7517	7,7217	-4,2814	7,5063	4,81	3,2884	3,1045	5,5864	-0,7169	3,0964	-3,4577	-3,3707	1,0166	-6,738	-4,3615	-1,7451	4,8674	-8,5136	4,0488	-0,8601	3,6848	0,049	3,3307	-4,9761	3,0328
BT483_BREAST	Luminal	3,928	7,8594	-1,5772	6,4925	5,1877	3,6946	2,9473	5,7423	0,274	1,4932	-3,3093	-2,9593	0,2364	-5,803	-5,442	-4,0525	3,4767	-6,5172	3,1025	-2,2836	3,841	2,7438	2,4982	0,567	1,7321
T47D_BREAST	Luminal	4,0403	7,1027	-4,6154	5,9563	5,6839	2,7983	3,0694	6,1806	-0,2538	1,864	-3,7466	-4,7206	-1,2839	-6,8569	-5,5029	-3,0438	5,0311	-7,333	3,6138	-3,0234	3,1613	2,081	4,2531	1,0596	0,8352
MDAMB175VII_BREAST	Luminal	4,2455	7,6449	-1,7768	5,6855	4,5853	3,4734	3,67	5,8217	0,2624	-0,2057	-3,4158	-2,1869	-3,6153	-7,0667	-4,8342	-1,8147	6,0222	-5,1318	4,2099	0,4366	4,3773	1,8252	3,8308	-1,3313	4,6249
ZR751_BREAST	Luminal	4,3153	6,7486	-1,4339	6,9532	4,5799	3,4601	2,7421	5,228	1,8034	2,3649	-2,0285	-3,2311	-0,6619	-4,7403	-3,8557	-2,5634	3,1855	-3,3739	3,977	1,3226	4,062	2,5719	3,4228	-0,0648	2,3135
MDAMB361_BREAST	Luminal	5,2617	7,9422	3,1555	7,3829	4,8253	2,1023	2,7789	5,4004	7,1862	5,3447	2,0425	-2,2178	1,4177	-2,486	-0,0714	-0,756	5,6164	-5,5347	3,7554	0,231	4,3575	2,1494	3,0418	0,3654	3,2521
HCC202_BREAST	Luminal	5,9451	7,0776	-1,7508	7,065	4,925	3,3869	2,7645	5,38	0,6653	-1,18	-4,6116	-3,3389	-0,9664	-6,3594	-4,8374	-3,2157	4,2737	-4,3981	3,6129	-4,6958	3,3326	2,6484	3,2131	1,0502	2,6058

Table S 2. EMT genes and breast cancer clusters and cell lines.

Protein	Clone/Ref	Species	Source	Dilution
P-cad	56 (610228)	Mouse	BD Transduction, USA	1:500
E-cad	24E10	Rabbit	Cell Signaling, USA	1:1000
N-cad	33-3900	Mouse	Invitrogen	1:1000
EpCAM	sc-25308	Mouse	Santa Cruz, USA	1:100
ZEB2	PA5-82094	Rabbit	Invitrogen	1:100
GRHL2	HPA004820	Rabbit	Sigma-Aldrich, USA	1:1000
AMPK	2532S	Rabbit	Cell Signaling, USA	1:1000
pAMPK	2535S	Rabbit	Cell Signaling, USA	1:1000
VIM	5741T	Rabbit	Cell Signaling, USA	1:1000
OCLN	5446P	Rabbit	Cell Signaling, USA	1:1000
ITGβ4	4707S	Rabbit	Cell Signaling, USA	1:1000
ΔNp63	Poly6190	Rabbit	BioLegend, USA	1:500
αTubulin	DM1A	Mouse	Sigma-Aldrich, USA	1:10000
GAPDH	sc-47724	Mouse	Santa Cruz, USA	1:1000
HSC70	sc-7298	Mouse	Santa Cruz, USA	1:8000

Table S 3. Detailed description of the experimental conditions for the antibodies used.

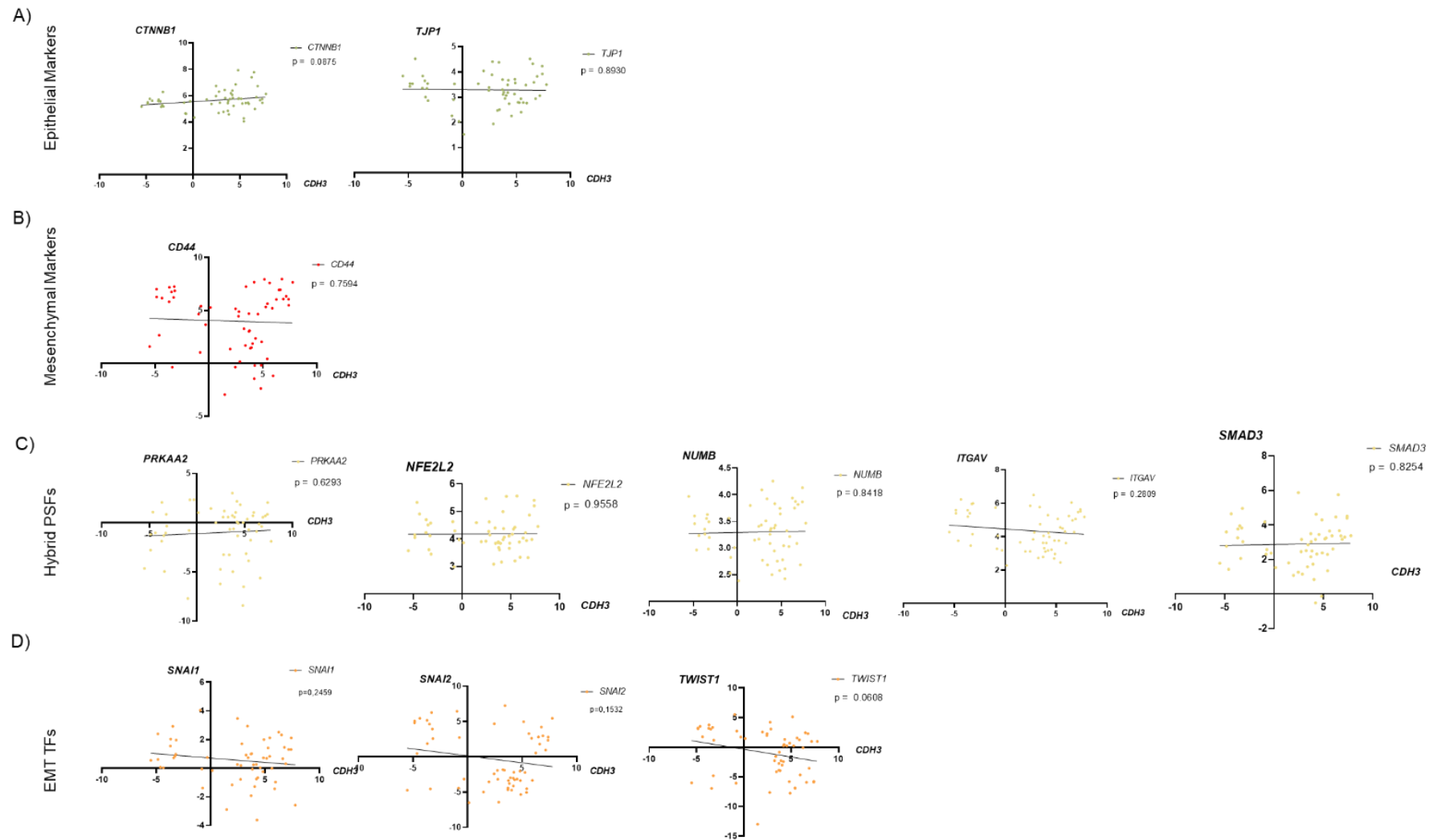


Figure S 1. Correlation between relevant EMT genes and CDH3. A) Correlation between Epithelial markers, such as CTNNB1 and TJP1, and CDH3. B) Correlation between CD44, a mesenchymal marker, and CDH3. C) Correlation between Hybrid PSFs, such as PRKAA2, NFE2L2, NUMB, ITGAV and SMAD3, and CDH3. D) Correlation between EMT TFs, such as SNAI1, SNAI2, and TWIST and CDH3.