



FMUP FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2018/2019

Diogo Paulo Moniz Garcia

The influence of the genetic and immunologic
context in the development of colorectal adenoma

março, 2019

FMUP



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Mestrado Integrado em Medicina

Área: Anatomia Patológica

Tipologia: Dissertação

Trabalho efetuado sob a Orientação de:

Doutor José Carlos Lemos Machado

E sob a Coorientação de:

Doutora Maria de Fátima Machado Henriques Carneiro

Trabalho organizado de acordo com as normas da revista:

The Journal of Pathology

março, 2019

FMUP

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Anatomia Patológica

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The influence of the genetic and immunologic context in the development of colorectal adenoma

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To all my friends and family,
for the unconditional support.

The influence of the genetic and immunologic context in the development of colorectal adenoma

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No conflicts of interest were declared.

ABSTRACT

The outcome for most patients with colorectal cancer remains relatively poor. There is renewed interest in immunotherapeutic options as there is a growing body of evidence supporting the existence of cancer immunosurveillance. The aim of the present study was to determine whether the hereditary or sporadic context has any influence in the relationship between immune microenvironment and tumor development.

The tumor-infiltrating cells of a total of 58 low-grade colorectal adenomas and 18 high-grade colorectal adenomas were examined and compared, using immunohistochemical staining for CD3, CD4, CD8, CD57, CD68 and FoxP3. Mutational burden was compared between sporadic adenomas and FAP adenomas, using Next Generation Sequencing.

FoxP3 and CD68 counts were significantly higher in low-grade dysplasia in patients with sporadic CRC ($p=0.0003$ and $p=0.0103$, respectively) when compared with corresponding lesions in FAP patients, whilst CD3, CD4, CD8 and CD57 counts were similar. In high-grade dysplasia lesions, CD4 and FoxP3 counts were significantly higher in patients with sporadic CRC ($p=0.0008$ and $p=0.0018$, respectively) when compared with corresponding lesions in familial adenomatous polyposis patients, whilst CD3, CD8, CD57 and CD68 were similar. Mutational burden was found to be significantly higher in familial adenomatous polyposis samples ($p=0.0040$).

This study shows that the immune microenvironment of sporadic and hereditary lesions is different. Sporadic lesions harbor higher tumor-infiltrating immune cell counts, namely immune suppressive Treg cells, and a lower

mutation burden. This might reflect a higher immune tolerance towards hereditary lesions, allowing for the development of lesions with a higher mutation burden, whilst having lower immune cell counts infiltrating the tumor lesions.

Keywords: Colorectal Neoplasms; Immunologic surveillance;
Immunohistochemistry

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in men and the second in women (1). Despite recent improvements, which include the introduction of new chemotherapeutic agents, the outcome for most patients remains relatively poor (2, 3). Therefore, additional treatment options beyond standard therapies (including surgery, chemotherapy and radiotherapy) are needed. There is renewed interest in immunotherapeutic options as there is a growing body of evidence supporting the existence of cancer immunosurveillance (ImSu) (4).

ImSu exerts an important influence in tumor progression and in the efficacy of anti-cancer therapies (5). Its success depends on the ability to detect neoantigens, frequently generated through mutations. Whether tumor-infiltrating lymphocytes represent the result of an inflammatory response that facilitates tumor progression, or a protective host response remains controversial (4).

The presence of a pronounced lymphocytic infiltration within the tumor is associated with improved survival (6-14) and several studies have indicated a relationship between the number of infiltrating tumor CD8⁺ T lymphocytes and an improved prognosis in CRC (15-18). Furthermore, the presence of high levels of tumor infiltrating memory T cells within CRC tissue has been associated with the absence of early metastatic spread and an improved disease-free and overall survival (19). Moreover, the amount of activated intraepithelial CD8⁺ lymphocytes infiltration in CRC is related to stage, which might indicate that immune reaction is more prominent in early stages of disease and might have a stage specific influence on survival (20, 21). It is also currently known that

immune dysregulation is already present in the early stages of the adenoma-CRC sequence (22).

There are multiple pathways in colorectal cancer pathogenesis, which include the chromosomal instability (CIN), microsatellite instability (MSI) and CpG island methylator phenotype (CIMP) pathways (23). CIN pathway is particularly suited for this study, since it includes, in most cases, a systematic progression through precursor lesions, which allows the study of the immune system activity in different stages of normal, precancerous and cancerous lesions of the colon. A key early mutation in the chromosomal instability (CIN) pathway is the mutation of adenomatous polyposis coli (APC) tumor suppressor gene. Familial adenomatous polyposis (FAP) is an autosomal dominantly inherited syndrome caused by germline mutations in the APC gene that accounts for approximately 1% of all CRCs (24). Affected individuals develop hundreds to thousands of adenomas in the colon and rectum at unusually young ages. If left untreated, one or more adenomas progress invariably to CRC.

Inherited genetic variants have been demonstrated to affect both baseline and induced host immune responses. In fact, anti-tumor immune responses are intrinsic characteristics, controlled, in part by the host's genome (25-27). However, such characteristics have yet to be systematically studied in hereditary models of cancer. A similar line of thought can be followed for somatic genetic variants. Given that sporadic lesions are expected to originate much later (10-30 years) than hereditary lesions, one would predict the baseline mutation burden to be higher in sporadic lesions. This in turn, should translate into higher immunogenicity in sporadic lesions when compared with hereditary lesions.

The aim of the present study, using sporadic and FAP-related colorectal adenomas as a model, was to determine whether the hereditary or sporadic context has any influence in the relationship between immune microenvironment and tumor development.

METHODS

Case selection

A total of 58 patients were selected from Centro Hospitalar Universitário São João, Porto, Portugal who had undergone either resection or biopsy (one single case). Twenty-seven FAP cases (14 male and 13 female), diagnosed between 1999 and 2015, and 31 sporadic cases (16 male and 15 female), diagnosed between 2016 and 2018 were used. Normal mucosa, low-grade dysplasia and high-grade dysplasia were analyzed. All lesions were located in the colorectum, with the exception of one lesion located in the duodenum.

Patients were matched for gender, but not for age, since CRC pathogenesis occurs at younger ages in FAP patients.

Immunohistochemical staining

Tissue sections were obtained from selected paraffin-embedded blocks, which were serially cut with a microtome (microm HM325) at 2 µm thickness and mounted on positively charged microscopic slides (Superfrost Plus™ from Thermo Scientific™). Amygdala was used as a positive control on each slide. Deparaffinization was accomplished with Ezprep at 72 °C over 20 minutes. Immunohistochemistry (IHC) staining was performed with the Ventana™ BenchMark ULTRA system. An optimization protocol, approved by a senior pathologist and a lab technician, was performed to achieve appropriate staining conditions. The protocol used is shown in Table 1.

Primary antibodies against CD3, CD4, CD8 and CD68 were added automatically by the Ventana™ machine with the aforementioned protocols, whilst FoxP3 and CD57 respective primary antibodies were added manually. OptiView™ Universal DAB detection kit was used.

Haematoxylin was used as a contrast agent (1 x 8 minutes) followed by Bluing reagent, an after-contrast agent (1 x 4 minutes).

After IHC, slide washing was performed with Ezprep (1x 10 minutes), detergent and running water (1 x 10 minutes until clean), followed by dehydration with ethanol [70% (1 x 10 minutes), 95% (1 x 10 minutes) and 100% (1 x 10 minutes)] and xylene (2 x 10 minutes). Finally, the slides were mounted with entellan and a protective slide.

Slide analysis

Haematoxylin and eosin (H&E) histological slides of each case were reviewed together with a senior pathologist to identify normal mucosa and adenomatous lesions. For each H&E case, a total of 6 IHC stained slides were obtained (1 slide for each different primary antibody). For each of the IHC slides a total of 8 high quality digital color images of the areas were obtained at 40x magnification using an Olympus BX 43 microscope (camera Olympus DP73) (4 images of normal mucosa and 4 images of the dysplastic lesion) for a total of 48 images per case. An example of normal mucosa can be observed in Figure 1-A, low-grade (Figure 1-B) and of high-grade dysplasia (Figure 1-C).

Each image representing a High-power field (HPF), corresponding to 0.06mm² was used to count the stained cells. Individual cells were manually counted by two observers in a double-blind setting, using ImageJ software

(National institutes of Health) that counts the clicks on each cell (Cell Counter plugin). The entire image was used for counting.

Inflammatory cells within areas of hemorrhage, necrosis, blood vessels, nerves or other large lymphoid aggregates were not quantified.

Cells were counted on each of the four images of both normal and adenomatous tissue, corresponding to a total area of 0.24mm², respectively.

Mutation burden analysis

In order to compare mutation loads between FAP and sporadic lesions, DNA was extracted from formaldehyde-fixed, paraffin-embedded (FFPE) tissue samples of both using the Applied Biosystems TM MagMAXTM FFPE DNA/RNA Ultra kit. Mutational burden was then analyzed using the OncoPrint TM Mutation Load Research Assay, which allowed the analysis of a total of 1.7 Megabases (Mb), of which 1.2Mb were exonic with an allele fraction sensitivity of 5%.

Statistical methods

For low-grade dysplasia, the differences in the number of stained cells/mm² between normal tissue and lesions were evaluated using unpaired Student's t-tests; the difference between the number of stained cells/mm² between hereditary and sporadic lesions was tested using unpaired Student's t-test.

Regarding high-grade dysplasia, due to the small sample size (n=9) and the presence of several outliers, non-parametric tests were used to infer the difference in the number of stained cells/mm² between normal tissue and lesions (Wilcoxon matched-pairs signed rank test); the difference between the number of

stained cells/mm² between hereditary and sporadic lesions was tested using a Mann Whitney test.

Mutational burdens were compared between sporadic patients and FAP patients, using unpaired non-parametric tests (Mann-Whitney test), due to the small sample size (n=5 and n=9, respectively) and the presence of several outliers.

RESULTS

Tumor-infiltrating immune cells

The tumor-infiltrating cells of a total of 58 low-grade dysplasias (Figure 2: A) and 18 high-grade dysplasias were examined and compared (Figure 2: B). Admitting a significance level of 5%, FoxP3 and CD68 counts were significantly higher in low-grade dysplasia in patients with sporadic CRC ($p=0.0003$ and $p=0.0103$, respectively) when compared with corresponding lesions in FAP patients, whilst CD3, CD4, CD8 and CD57 counts were similar. In high-grade dysplasia lesions, CD4 and FoxP3 counts were significantly higher in patients with sporadic CRC ($p=0.0008$ and $p=0.0018$, respectively) when compared with corresponding lesions in FAP patients, whilst CD3, CD8, CD57 and CD68 were similar. Examples of low-grade adenomas from both sporadic and FAP patients can be observed in Figure 3, whilst examples of high-grade adenomas can be observed in Figure 4. The statistical significance analysis is shown in table 2.

Mutational burden

In order to explain the higher cell counts observed in sporadic lesions (Figure 2: A and B), mutation burden was studied in both sporadic and FAP samples. Given the later onset of the former, a higher mutation burden would be expected, which could translate into a higher immunogenicity and, as such, help explain the higher cell counts observed in the respective tumor-immune infiltrates.

However, mutation burden (Figure 2: C) was found to be significantly higher in FAP samples ($p=0.0040$).

DISCUSSION

Accumulating evidence supports the importance of the host's immune system on the development of cancer (28-34), which may offer powerful prognostic tools and guide the need for systemic therapy. ImSu can not only eliminate tumors but also select variant tumor cells that resist the ImSu mechanism, a process called 'immunoediting' (35, 36). Higher densities of tumor-infiltrating lymphocytes are generally associated with improved clinical outcome (37). In particular, higher densities of CD3+ T cells and CD8+ T cells have been associated with longer disease-free survival and/or improved overall survival. Therefore, the role of the adaptive immune response is becoming increasingly appreciated. On the other hand, there is experimental evidence supporting the idea that the innate immune system can promote tumor development through inflammation-dependent mechanisms (38, 39). Therefore, the precise analysis of the tumor microenvironment by pathologists may be essential for future clinical implementation and better patient management.

Previous studies have examined the independent prognostic effect of tumor-infiltrating immune cells in CRC models. We aimed to examine the implication of the hereditary context of FAP in the tumor-infiltrating cells of pre-cancerous lesions.

The current study demonstrates that the hereditary context of FAP results in significantly different phenotypes of tumor-infiltrating immune cells. In sporadic low-grade dysplasia, a significantly higher number of FoxP3 and CD68 stained cells was observed, whilst CD4 and FoxP3 cell counts were significantly higher in sporadic high-grade dysplasia lesions.

Regulatory T cells (Treg) are a subpopulation of CD4+ T cells that express the transcription factor forkhead box protein P3 (FoxP3) and hold a key role in maintaining self-tolerance by suppressing the activation and function of self-reactive lymphocytes (40). In cancer infiltrates, however, Treg cells suppress the induction and proliferation of effector cells to impair antitumor immunity, contributing to a poor clinical outcome in cancers with abundant Treg infiltration. However, its role in CRC is controversial, having a potentially positive impact by controlling cancer-driving inflammation, whilst, also, promoting tumor progression by impeding specific immune responses (41), and, in fact, higher densities of Treg cells have been associated with better survival in CRC (42, 43), which might explain the higher counts of FoxP3 stained cells in sporadic lesions, observed in both low grade and high grade dysplasia in the current study (44).

Tumor-associated macrophages (TAMs), stained with CD68, have been associated with a pro-tumor phenotype in both primary tumors and metastasis, by promoting increased growth, angiogenesis, metastases, immunosuppression and poorer differentiation (45, 46). Its role in CRC, however, is not as straightforward, and, in contrast to most other malignancies, in CRC, increased macrophage infiltration is associated with better patient prognosis (13, 47-52). It is likely that infiltrating monocytes are primed in the tumor micro-environment to exert either anti or pro tumorigenic characteristics, depending on environmental cues that they receive, with most studies on colon cancer supporting a tumor-inhibiting role of macrophages, with higher infiltrating densities being related with decreased recurrence, diminished metastases and increased patient survival (47-49, 52-54), which might help to explain why significantly higher counts were identified in the current study in sporadic lesions, specifically in low grade lesions,

which might imply that its anti-tumor effect might be lost during the progression from low grade to high grade lesions.

Tumor-selective infiltration of CD4+ T cells might be explained by the role played by CD4+ cells during the primary antigen-specific response in imprinting CD8+ T cells with the ability to develop into long-living memory cells (55), which might explain the higher counts observed in this study in high grade dysplasia lesions of sporadic patients.

Despite having a later onset, sporadic samples were found to carry a lower mutation burden when compared with FAP samples, which might reflect a higher immune tolerance towards mutations in a hereditary context.

This study shows that sporadic lesions harbor higher tumor-infiltrating immune cell counts and lower mutation burdens, which might both reflect a higher immune tolerance towards hereditary lesions, allowing for the development of a higher mutation burden, whilst having lower immune cell counts infiltrating the tumor lesion.

Table 1: IHC protocol

Antibody	Type/Clone of primary Antibody	CC1 solution's exposure time (minutes)*	Option solution's exposure time (minutes)**	Antibody's exposure time (minutes)	Staining pattern
<i>Anti-CD3</i>	Anti-CD3 (2GV6) Rabbit monoclonal primary antibody	40 (95 °C)	Not used	20 (0.4µg/ml)***	Cytoplasmic and membranous
<i>Anti-CD4</i>	Anti-CD4 (SP35) Rabbit monoclonal primary antibody	40 (95°C)	4	32 (2.5µg/ml)***	Membranous
<i>Anti-CD8</i>	Anti-CD8 (SP57) Rabbit monoclonal primary antibody	24 (100°C)	4	20 (0.35µg/ml)***	Membranous
<i>Anti-CD57</i>	Abcam monoclonal mouse AB 187274	40	8	28 (1:300 dilution)	Cytoplasmic and membranous
<i>Anti-CD68</i>	Anti-CD68 (KP-1) Primary Antibody	16 (100 °C)	8	16 (0.4 µg/ml)***	Cytoplasmic and membranous
<i>Anti-FoxP3</i>	Polyclonal anti-FoxP3 rabbit NB 600-245	40	4	40 (1:300 dilution)	Nuclear

*CC1: Cell conditioning 1 is a prediluted solution used as a pretreatment step in IHC tissue processing reactions carried out on Ventana™ BenchMark.

**Prediluted solution ready-to-use in order to diminish background staining.

***Prediluted antibody solution

Table 2: Statistical Significance Analysis

Low grade dysplasia (FAP versus SPO)		FAP cell counts (mean)	Sporadic cell counts (mean)	P-value	Statistical significance
	CD3	1397	1353	0.8178	ns
	CD4	1152	1351	0.3042	ns
	CD8	438	384.3	0.4033	ns
	FoxP3	100.3	246.8	0.0003	***
	CD57	94.92	91.13	0.9298	ns
	CD68	538	842.9	0.0103	*
High grade dysplasia (FAP versus SPO)		FAP cell counts (median)	Sporadic cell counts (median)	P-value	Statistical significance
	CD3	925	1050	0.4363	ns
	CD4	675	1742	0.0008	***
	CD8	354.2	412.5	0.6665	ns
	FoxP3	4.167	137.5	0.0018	**
	CD57	20.83	25	0.5560	ns
	CD68	475	900	0.1135	ns

* p<=0.05; ** p<=0.01; ***p<=0.001; ****p<=0.0001; ns p>0.05

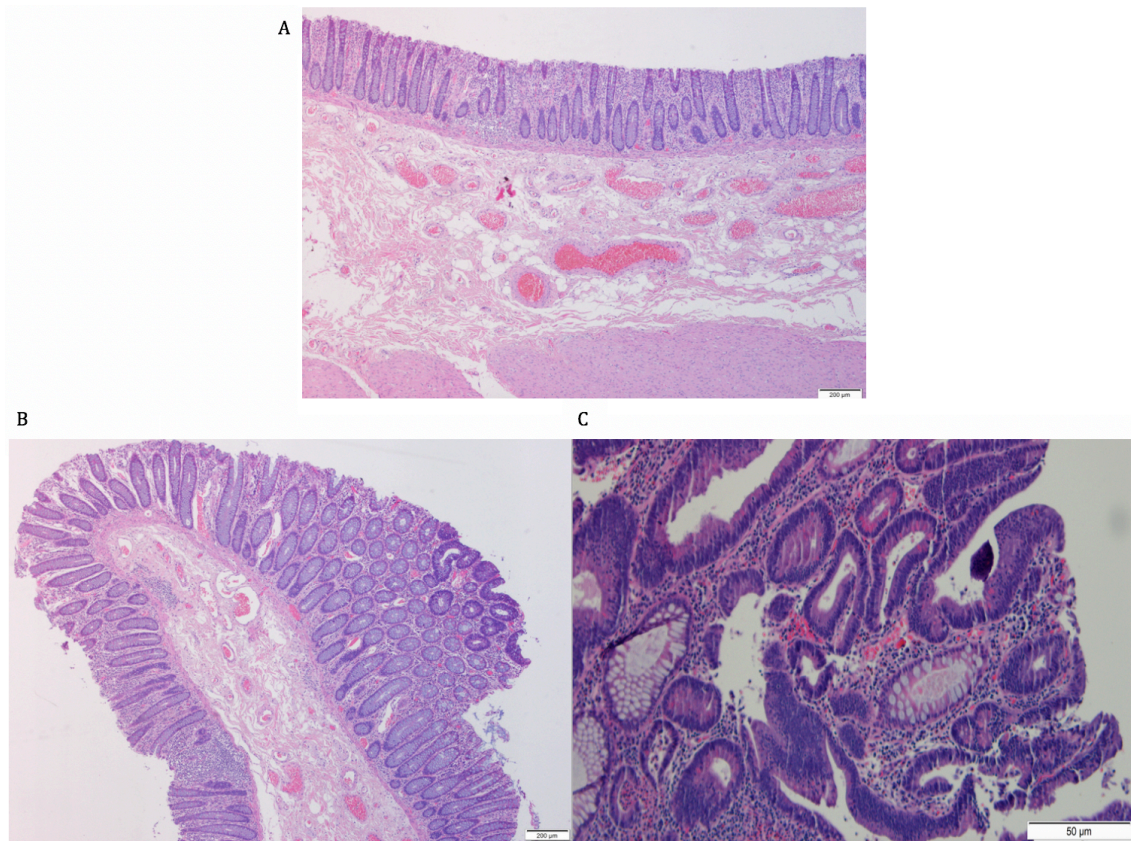
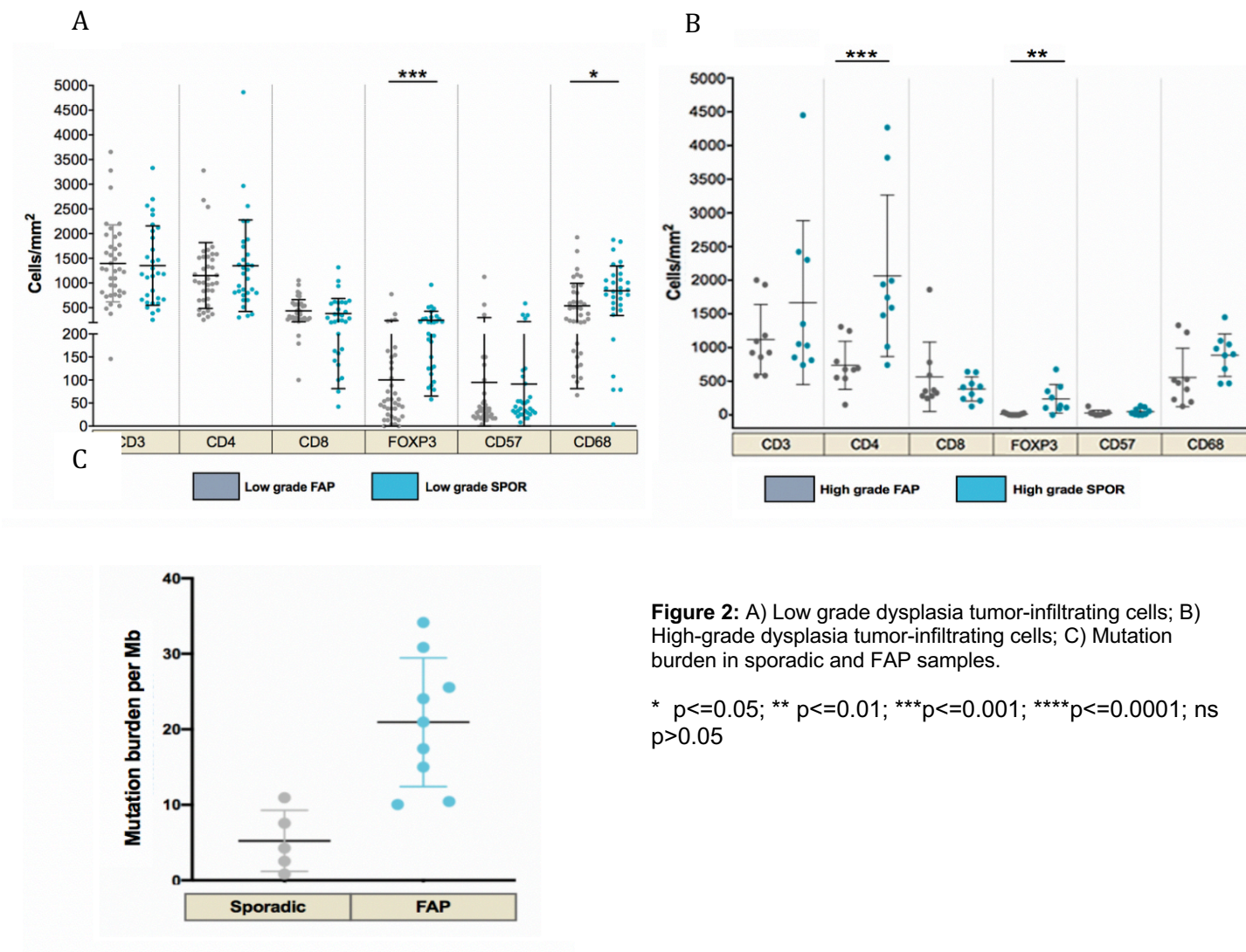
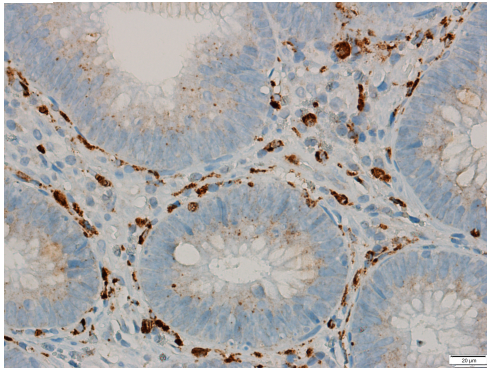


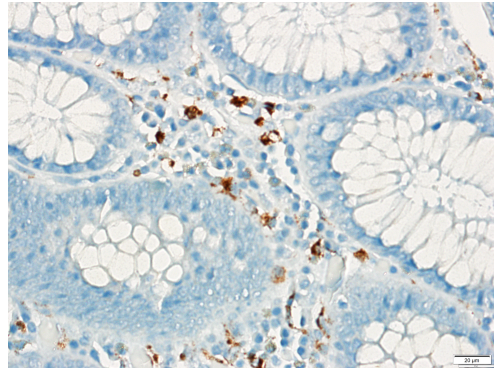
Figure 1: A: Normal mucosa in a FAP patient (x4). B: Low-grade dysplasia in a FAP patient (x4). C: High-grade dysplasia in a FAP patient (x10).



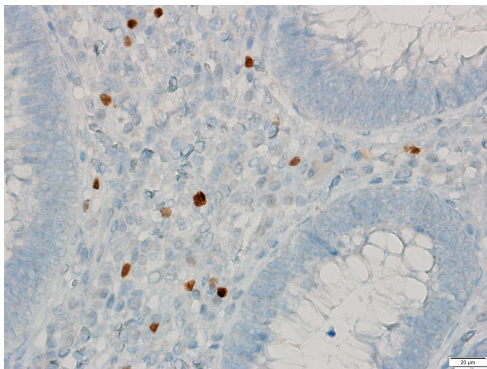
A



B



C



D

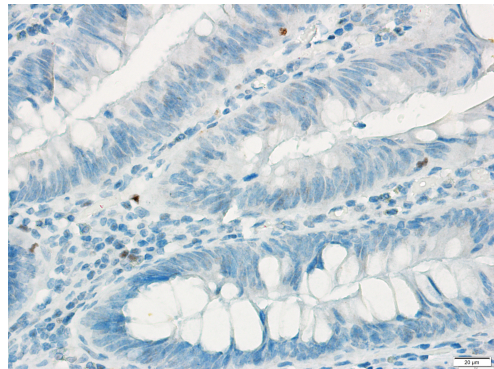
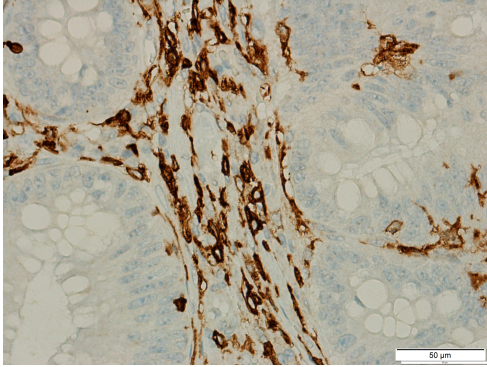
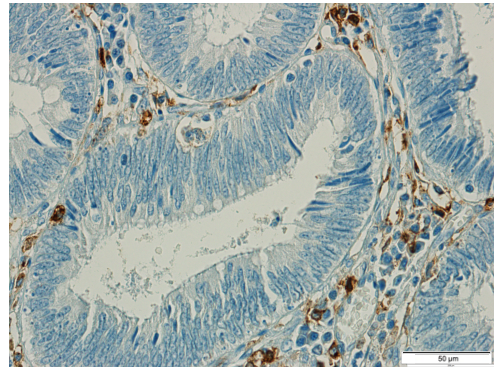


Figure 3: Low-grade dysplasia (x40): CD68 stained cells in sporadic (A) and FAP (B) adenoma; FoxP3 stained cells in sporadic (C) and FAP adenoma (D).

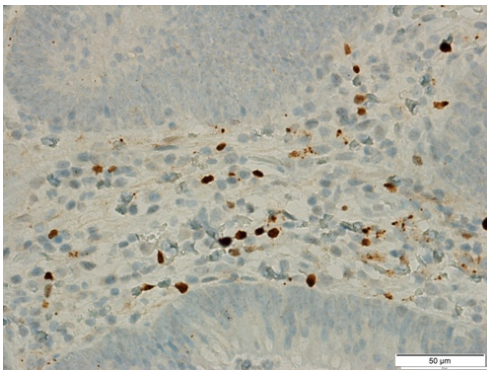
A



B



C



D

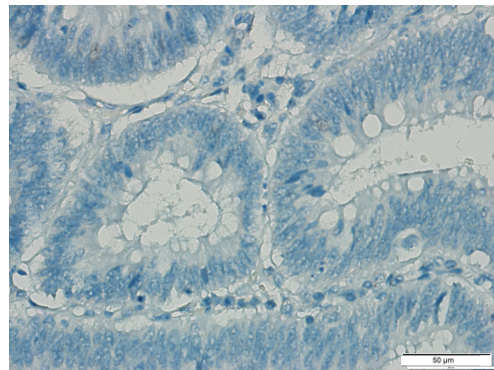


Figure 4: High-grade dysplasia (x40): CD4 stained cells in sporadic (A) and FAP (B) adenomas; FoxP3 stained cells in sporadic (C) and FAP adenomas (D);

Acknowledgements:

I would like to sincerely thank all the fellows, colleagues and collaborators who contributed to this work.

Author contribution statement

DG was responsible for data collection, data analysis, data interpretation, literature search, generation of figures and writing of the manuscript. LS was responsible for data collection, data analysis, data interpretation and literature search. SM was responsible for data collection, data analysis and generation of figure 2:A. FC and JCM were responsible for study design, data analysis, data interpretation and writing of the manuscript.

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Author Guidelines

1 MANUSCRIPT PREPARATION AND SUBMISSION

All manuscripts must be submitted using our online system at <http://mc.manuscriptcentral.com/jpath>. There are no submission or page charges.

Every submission should conform to one of the categories listed below. Use the link from each category to obtain a brief mini-guide of manuscript requirements and format. Consult the detailed guidelines below (also linked to from each mini-guide) for more detail on each aspect of manuscript preparation.

- **Original Articles**

Original articles are our main type of primary publication. They are up to 4000 words in length from beginning of Introduction to end of Discussion with up to 6 figures. Further information can be found [here](#)

- **Brief Definitive Reports**

Brief Definitive Reports provide a forum for the rapid publication of highly significant and timely advances. They are up to 1500 words in length from beginning of Introduction to end of Discussion with up to 4 tables and figures. Further information can be found [here](#)

- **Reviews**

Scholarly critical reviews of important subjects within the Journal's scope. They are usually no more than 4000 words. Further information can be found [here](#).

- **In Brief**

Mini-reviews highlighting topical subjects. These are usually invited but suggestions are welcomed. Typically 1000 words Further information can be found [here](#)

- **Commentaries**

Brief reviews placing recently published primary research from the Journal into context. These are usually less than 2000 words. Further information can be found [here](#)

- **Perspectives**

Overview articles highlighting novel, debatable, or highly topical aspects of pathology research. These are usually less than 2000 words. Further information can be found [here](#)

2 AIMS AND SCOPE

The *Journal of Pathology* and the companion *Journal of Pathology: Clinical Research* serve as translational bridges between basic biomedical science and clinical medicine with particular emphasis on, but not restricted to, tissue based studies.

The focus of the *Journal of Pathology* is the publication of studies that further our understanding of the pathophysiological and pathogenetic mechanisms of human disease. Studies that bring pathological, biological and or clinical sense to genomic discoveries are particularly welcomed.

Studies with a predominantly clinical focus including those focusing on diagnostic, prognostic and predictive markers, and biomarker discovery and validation, will be more appropriate for the companion *The Journal of Pathology: Clinical Research*.

The Journal welcomes investigative studies on human tissues, in vitro and in vivo experimental studies, and investigations based on animal models with a clear relevance to human disease, including transgenic systems. In general, hypothesis driven studies that appropriately employ multiple investigative techniques are preferred over those that rely on a single methodology.

As well as original research papers, the Journal seeks to provide rapid publication in a variety of other formats, including editorials, review articles, commentaries and perspectives and other features, both contributed and solicited. In all cases, contributions are subjected to strict peer review.

Purely descriptive papers, including those on diagnostic pathology (including case reports and prognostic papers without mechanistic and experimental insights) are not considered central to the Journal's purpose.

Correspondence relating to papers published in the Journal will be considered, but only if of general interest. Such correspondence may be published in online versions only, at the Editor's discretion.

In determining content, the primary considerations are excellence, relevance and novelty. As a journal of **The Pathological Society**, it seeks to reflect the broad scientific interests of the Society's membership but its ethos, authorship, content and purpose are those expected of a leading publication in the international scientific literature.

3 THE REVIEW PROCESS

For further detail, please see this Editorial: **[A guide to the Editorial processes at The Journal of Pathology](#)**. *J Pathol* 2009;**142**:1-6.)

On receipt, manuscripts are assessed by the Editor-in-Chief, a Senior Editor and at least one Associate Editor. At this point, those outside our scope or quality criteria are returned to the authors, typically within three working days.

Manuscripts selected for external review are assigned to an Associate Editor, who solicits two or more expert reviewers. A similarity search engine (iThenticate®) is used to detect plagiarism and duplicate publication.

Authors may suggest up to five external referees, but the Editorial team will not be bound by these nominations. Likewise, authors may state why a named individual may be inappropriate as a referee, again without binding the Editorial team.

The Reviewers' and Associate Editor's views are used by the Editor-in-Chief (or a Senior Editor) in reaching a decision, usually within three weeks of submission. The decision letter will indicate whether the manuscript is accepted, should be revised or is rejected. Minor and major revisions are expected within four weeks and three months of this decision, respectively. Invitation to submit a revision does not imply that acceptance will follow.

If authors dispute a decision, they should offer a compelling rebuttal, initially by writing to the Editor-in-Chief

3.1 PRE-SUBMISSION ENQUIRIES

Given our rapid initial review process, **we do not accept pre-submission enquiries for original research papers**. For guidance on “fit” to the Journal, authors should read these Guidelines and examine recent published issues.

We are happy to hear from potential authors of all categories of **review article** (including Commentaries and Perspectives). Contact the Editorial team via eic@jpathol.org

3.2 CONTACTING THE EDITORIAL TEAM

For technical and administrative matters, please contact The Managing Editor (Mrs Nicky Cotterill) via managing_editor@jpathol.org

For scientific matters, the senior Editorial team can be contacted via eic@jpathol.org

Author Guidelines

4 DETAILED NOTES ON MANUSCRIPT PREPARATION AND SUBMISSION

4.1 The cover letter

This must explicitly state that the manuscript comprises original unpublished work and is not under consideration for publication elsewhere; any prior submissions that might be regarded as redundant or duplicate publication must be declared, including previously published or presented abstracts. The corresponding author must confirm that all authors have agreed with the submission in its present form. Any other manuscripts by the authors that are relevant to the submitted manuscript, and which are currently under review elsewhere or in press, must be listed and uploaded as ‘Additional files for review but NOT for publication’.

4.2. General points

Format the word processing document as double spaced A4 pages with an additional space between paragraphs and margins of at least 2 cm all round. Use a 12-pt standard font such as Times, Helvetica or Arial (with Symbol for special characters). Do not use line numbering, but include page numbers in the header or footer, aligned right. Use consistent, preferably UK English spelling.

4.3 Manuscript title

This should be clear, simple and concise; long titles lack impact. Please remember that many readers will only scan titles, so they should reflect the message of the paper and catch the readers’ attention.

4.4 A short running title

This must be 75 characters or less, including spaces, and reflect the main title and content of the manuscript.

4.5 List of authors

Authorship credit should be based only on 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; 3) final approval of the version to be published. Conditions 1, 2 and 3 must all be met. Acquisition of funding, the collection of data or general supervision of the research group, by themselves, do not justify authorship. All others who contributed to the work but are not authors should be named in the Acknowledgements section.

A statement outlining the specific contribution of each author to the manuscript and the work reported in it must appear after the acknowledgements section (**see below**).

If you are submitting a REVISION and the authors have changed from the original submission, for example: if additional authors are added [or removed] then agreement of all authors is mandatory, with justification and explanation required within the cover letter, submitted via the ScholarOne system.

4.6 Full affiliations of all authors

Include the name of the department(s) and institution(s) to which the work should be attributed. Append the corresponding author(s) full postal address, phone number and email address.

4.7 Conflict of interest statements

Authors must disclose all financial and personal relationships that might bias their work; to prevent ambiguity, a conflict of interest statement must appear on the manuscript title page, detailing any conflicts (or the absence thereof) for each author. There is a recent Editorial on this subject (Hall PA, Poulson R, Wixon J. **How does the *Journal of Pathology* deal with conflict of interest?** J Pathol 2009; 219: 396-399).

Please see this accompanying **document** for details of our guidance on conflict of interest, with examples.

4.8 Word count (from beginning of Introduction to end of Discussion)

Concise articles make a greater impact than long ones and are less likely to be delayed by editing to a suitable length. Full articles should be no more than 4000 words from the beginning of the Introduction to the end of the Discussion. Review articles and special features may occasionally exceed this limit by arrangement with the Editor-in-Chief.

Online Supporting Information can be used for presenting additional material. However, authors must ensure that the main manuscript contains sufficient information to make the study intelligible without referring to the Supporting Information. For example, while it is not acceptable to shorten a manuscript by placing the entire Materials and Methods in Supporting Information, methodological details (e.g. PCR primer sequences) should go in Supporting Information.

4.9 Abstract (not structured and no more than 300 words)

Following the title page(s), the next page should carry an unstructured prose abstract of 300 words or less. It should clearly convey the purposes of the study, and the main procedures, findings and conclusions. It should be understandable without reference to the rest of the paper, and contain no citation to references in the reference list. Only **standard abbreviations** as listed below are permitted.

4.10 Keywords (3 to 10)

Below the abstract, authors should provide and identify as such 3 to 10 keywords or short phrases to assist indexing the article and that may be published with the abstract. MESH headings are a useful guide for authors in considering keywords. In addition, authors should examine the text of their title, abstract and keywords since any term not employed in these sections cannot be employed as a search term to reliably identify the work using standard search engines (including PubMed).

4.11 Manuscript structure

Research articles are divided into sections with the headings: Abstract, Introduction, Methods, Results and Discussion. Long articles may need subheadings (especially within the Results and Discussion) to clarify their content. The sections should not be numbered. Other types of articles, such as reviews and commentaries, still need a title and abstract and should adhere as closely as possible to these guidelines.

Authors are encouraged to consult reporting guidelines relevant to their specific research design, in particular:

- Reports of tumour studies should be fully compliant with the REMARK guidelines <http://www.ncbi.nlm.nih.gov/pubmed/16106022> and this should be outlined in the cover letter.
- For all types of microarray analyses, follow the MIAME guidelines <http://www.mged.org/Workgroups/MIAME/miame.html>.
- Authors should note that it is mandatory to make the array data available. This should preferably be via a public database such as the GEO or Array Express databases prior

to submission (release embargos until acceptance are permitted), quoting the accession number/s in your manuscript. An alternative is to make the data available via the *Journal of Pathology* website as Supporting Information.

- For reports of randomised controlled trials, follow the CONSORT statement <http://www.consort-statement.org>.
- For reports of studies involving animals, follow the ARRIVE guidelines and consider following the MINPEPA guidelines.

4.11.1 Structure of Commentaries, Perspectives and Reviews

These have greater flexibility than standard original articles, but a concise clear style is essential. While most are invited, the editorial team is happy to receive suggestions and outlines of possible contributions of these kinds. All will undergo peer review.

Commentaries are typically brief reviews (2 to 3 journal pages) placing a paper (or group of papers) in the Journal into a broader context. They should be focused, pithy, and accessible to a broader audience than the primary papers. Diagrams are often useful to illustrate concepts and ideas.

A **Perspective** is similar in form and length, but will have a broader remit and is not necessarily linked to a paper in the Journal. It may be polemical or provocative.

A **Review article** can range in length from short items (mini-review) through to very detailed, fully referenced contributions. However, a review should be focused in nature and not simply a literature survey: it must have a point!

4.12 Introduction

Authors should state the purpose of the article and summarise the rationale for the study or observation, providing relevant background. The length of the Introduction is not specified but it should be in proportion to the rest of the manuscript and sufficiently comprehensive to allow a non specialist to understand the setting of the work. Give only strictly pertinent references and avoid the inclusion of data or conclusions from the work being reported. The aims of the study and hypothesis being tested should be stated clearly and objectively.

4.13 Materials and methods

4.13.1 General aspects of Materials and Methods

Clearly describe your selection of the observational or experimental subjects (patients or laboratory animals, including controls). Identify the age, sex and other important characteristics of the subjects where appropriate. As the relevance of such variables as age, sex and ethnicity to the object of research is not always clear, authors should explicitly justify them when they are included in a study report. The guiding principle should be clarity about how and why a study was performed in a particular way. Authors should avoid terms such as 'race' which lacks precise biological meaning and use alternative descriptors such as 'ethnicity' or 'ethnic group' instead. Authors should specify carefully what the descriptors mean and tell exactly how the data were collected.

Identify the methods, apparatus (give the manufacturer's name and address in parentheses) and procedures in sufficient detail to allow other workers to reproduce the results. Give references to established methods, including statistical methods (see below); provide references and brief descriptions for methods that have been published but are not well known; describe new or substantially modified methods, give reasons for using them and evaluate their limitations. Identify precisely all drugs and chemicals used, including generic name(s), dose(s) and route(s) of administration

Studies using antibodies must define them using catalogue or clone numbers for the primary antibodies used and their supplier. Details of antigen retrieval, antibody dilution, secondary layers, fluorophores or colour reactions must be provided. Validation steps and controls must be explained.

You may wish to consider using Research Reagent IDs (RRID) which are available at the [Resource Identification Portal](#).

Studies using cell lines must explain how each was obtained and describe steps taken to prove their identity. A minimum requirement is to check the **ICLAC** and/or **NCBI** databases to ensure that the cell lines are not misidentified.

Studies on biomarkers and/ or prognostic and predictive factors must describe the patient selection criteria and provide a detailed breakdown of the cohort(s) included in the study. Reports of randomised clinical trials should present information on all major study elements, including the protocol (study population, interventions or exposures, outcomes and the rationale for statistical analysis), assignment of interventions (methods of randomisation, concealment of allocation to treatment groups) and the method of masking (blinding).

Studies employing high throughput molecular methods, such as microarray-based expression profiling and microarray-based comparative genomic hybridisation, should provide accurate information about percentage of the cells of interest in the samples, method of nucleic acid extraction, RNA or DNA amplification methods, details of the platform used and the analysis methods employed. We would anticipate that data from high throughput analyses, whether array based or otherwise should be made available via a suitable database or via files in online **Supporting Information**. Relevant clinical information should accompany this data.

Important information that is not essential for the understanding of the manuscript may appear as **Supporting Information** online. Examples of this would include PCR primer sequences and extraneous experimental detail. As a guide, information required to understand what was done and how, should be in the manuscript. Detail required to fully replicate the experiments can appear as Supporting Information. Please note that it is unacceptable to move the entire Materials and Methods section to Supporting Information.

Reliable studies of clinical samples require high quality samples and we anticipate that authors will document BRISQ Tier one variables (see Table 1 in **Moore *et al.* Biospecimen Reporting for Improved Study Quality, Cancer Cytopathology 2011;119:92-101**)

4.13.2 Ethical issues

An explicit statement describing the ethical background to the study must be included in the Materials and Methods. Ethics committee or institutional review board approval should be cited, with relevant reference numbers and dates. Indicate whether procedures involving human subjects were in accordance with the Helsinki Declaration of 1975, as revised in 1983. When reporting experiments on animals, indicate which institutional or national guidelines or law on the care and use of laboratory animals was followed. Authors should be mindful of ethical standards such as those in "Guidelines for the welfare and use of animals in cancer research"; (Workman, Aboagye, Balkwill *et al.* *Br J Cancer* 2010; **102**: 1555-1577)

Patients have a right to privacy that should not be infringed without informed consent. Identifying information should not be published in written descriptions, photographs and pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) gives written informed consent for publication. Informed consent for this purpose requires that the patient be shown the manuscript to be published. Identifying details should be omitted if they are not essential but patient data should never be altered or falsified in an attempt to attain anonymity. Complete anonymity is difficult to achieve and **informed consent should be obtained** if there is any doubt. For example, masking the eye region in photographs of patients is inadequate protection of anonymity.

The Journal recognises that the requirements and legislation pertaining to all aspects of ethical review may vary between different jurisdictions. If there are any legitimate concerns raised by reviewers or editors about any aspect of the ethical issues of the study that cannot be satisfactorily resolved, the Editor-in-Chief reserves the right to decline to publish the manuscript.

4.13.3 Statistics and bioinformatics

Data sets should be made publicly available either via public repositories or online **supporting information**. Authors should provide complete data annotation including all the clinical data reported in their manuscripts using the same identifiers reported in the paper. Authors are encouraged to create a reviewer secured access link to their data during the review process.

Describe statistical and bioinformatic approaches for both data processing and analysis with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, quantify findings and present them with appropriate indicators of error or uncertainty (such as standard error or confidence intervals). Sample size should always be indicated. References for the design of the study and statistical methods should

usually be to standard works (with pages stated) but citation to the papers in which the designs or methods were originally reported may sometimes be appropriate. Restrict tables and figures to those needed to explain the argument of the paper and to assess its support. Use graphs as an alternative to tables with many entries: do not duplicate data in graphs and tables. Data underlying figures and graphs should not be included in the main body of the paper, but may be provided in **supporting information**. Avoid non-technical uses of technical terms in statistics, such as 'random' (which implies a randomising device), 'normal', 'significant', 'correlations' and 'sample'.

Open source and publicly available statistical/ bioinformatic software packages, which allow other workers to reproduce the analysis performed, are to be preferred. If using a self-developed algorithm, this tool (a workflow of usage and a short documentation) should be made available on request or presented as **supporting information**, where possible. For high throughput sequencing we recommend consideration of the minimal information to be supplied as outlined by MGED (<http://www.mged.org/minseql/>).

Data derived from array-based experiments must be interpreted cautiously and validated using independent laboratory-based technology. Such validation would ensure that these are not outliers or noise. Detailed information should be given about the annotation framework used by the authors. Versions or releases of databases, sequences and software packages should be also reported.

The *Journal of Pathology* expects that data supporting the results in the paper will be archived in an appropriate public repository. Whenever possible the scripts and other artefacts used to generate the analyses presented in the paper should also be publicly archived. Exceptions may be granted at the discretion of the editor for sensitive information such as human subject data. Authors are expected to provide a data accessibility statement, including a link to the repository they have used, to accompany their paper.

4.14 Results

Present your results in logical sequence in the text, tables and illustrations. Do not repeat in the text all the data in the tables or illustrations; emphasise or summarise only important observations. Figures and tables should be clearly referred to in the manuscript. It is generally the case that interpretation should be reserved for the discussion.

4.15 Discussion

Emphasise the new and important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material given in the Introduction or the Results section. Include in the Discussion section the implications of the findings and their limitations, including implications for future research. Relate the observations to other relevant studies including those that may conflict with the data you present.

Link the conclusions with the goals of the study but avoid unqualified statements and conclusions not completely supported by the data. In particular, authors should avoid making statements on economic benefits and costs unless their manuscript includes economic data and analyses. Avoid claiming priority and alluding to work that has not been completed. State new hypotheses when warranted, but clearly label them as such. Recommendations, when appropriate, may be included. References to unpublished data should not be included.

4.16 Acknowledgements

List all contributors who do not meet the criteria for authorship criteria (See Section 4.5) Groups of people who have contributed materially to the paper but whose contributions do not justify authorship may be listed under a heading such as 'clinical investigators' or 'participating investigators' and their function or contribution should be described. The acknowledgements must also contain a statement defining funding sources. This should include source(s) of support in the form of grants, equipment, or drugs, all of these should be clearly and fully defined, including sources of materials, reagents or other resources as gifts.

4.17 Author contributions statement

A statement outlining the specific contribution of each author to the manuscript and the work reported in it must appear eg, study design, data collection, data analysis, data interpretation, literature search, generation of figures, writing of the manuscript. If all authors contributed

equally, please state this. An example of the style we would prefer is: ABC and EF conceived and carried out experiments, JKL and PQ conceived experiments and analysed data. MN carried out experiments. All authors were involved in writing the paper and had final approval of the submitted and published versions.

4.18 List of abbreviations (optional)

The Journal abbreviates all units of measurement for physical and chemical quantities according to standard metric usage (International System of Units (SI) base units and supplementary units). Standard abbreviations for countries (UK, USA etc), organisations (WHO, CRUK, NIH etc) and similar may be used. A list of **standard abbreviations and symbols** is provided these may be used singularly, or in combination, and do not need to be spelt out. For all other abbreviations, the full term for which an abbreviation stands should precede its first use in the text. A list of such non-standard abbreviations may be added after the acknowledgements section. Avoid abbreviations in the title and abstract

4.18.1 Units of Measurement

Measurements of length, height, weight and volume should be reported in metric units (metre, kilogram or litre) or their decimal multiples. Temperatures should be given in degrees Celsius. Blood pressures should be given in millimetres of mercury. All haematological and clinical chemistry measurements should be reported in the metric system in terms of the International System of Units (SI). Concentrations should usually be given as molarity (mol/L, abbreviated M).

4.18.2 Gene names

Human gene names should be upper case and italic and mouse gene names lower case and italic; protein names should be in plain type. Follow HUGO gene nomenclature for human genes, at: <http://www.genenames.org/>

Note differences between human and rodent gene, name conventions, and for mouse and rat gene nomenclature please follow the guidelines at:

<http://www.informatics.jax.org/nomen/>

For other species authors should take care to use standard internationally accepted nomenclature and we suggest that authors consult Entrez Gene (<http://www.ncbi.nlm.nih.gov/gene>) to check the correct nomenclature for their species, as it lists the different symbols for each known gene.

4.19 List of supplementary material online (when required)

If used, a list of the files containing supplementary materials and methods, supplementary figure legends, supplementary figures, tables and movies must be provided after the figure legends in the main text file. A concise title should be provided for each file, and the files should follow the guidance in 4.24 Supplementary material.

4.20 Reference list (in the correct format)

References should be numbered consecutively in the order in which they are first mentioned in the text. Identify references in text, tables and legends by Arabic numerals in square parentheses. References cited only in tables or figure legends should be numbered in accordance with the sequence established by the first identification in the text of the particular table or figure. All references should be complete and accurate.

List all references cited in the main text reference list. If a source is cited only in supplementary materials place that reference at the end of the main text list and with an asterisk, eg. *99. Smith A, Jones B, At the end of the references section write *Cited only in supplementary materials.

Avoid using abstracts as references. References to papers accepted but not yet published should be designated as 'in press'; authors should obtain written permission to cite such papers as well as verification that they have been accepted for publication. Copies of any papers cited as 'in press' must be included in the submission. Avoid using unpublished observations and information from manuscripts submitted but not accepted.

Avoid citing a 'personal communication' unless it provides essential information not available from a public source, in which case the name of the person and date of communication should

be cited in parentheses in the text. Authors must supply written permission and confirmation of accuracy from the source of a personal communication.

The Journal's reference style is modified Vancouver used by the NLM in Index Medicus. For articles with more than 3 authors, only the first 3 authors should be listed, followed by *et al.* The titles of journals should be abbreviated according to the style used in Index Medicus. Title abbreviations can be checked using the PubMed Journals database: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=journals&term=>. Articles published online but not yet assigned to an issue may be cited using the DOI (see example 4). Online citations should include the date of access. Examples of our reference style are:

Journal article from an issue

Going JJ, Moffat DF. Escaping from Flatland: clinical and biological aspects of human mammary duct anatomy in three dimensions. *J Pathol* 2004; **203**: 538–544.

Journal article in advance of inclusion in an issue

Strizzi L, Bianco C, Hirota M, *et al.* Development of leiomyosarcoma of the uterus in MMTV-CR-1 transgenic mice. *J Pathol* 2006; DOI:10.1002/path.2083

Chapter in an edited book

Watson F, Herrington CS. Blotting techniques: Methodology and Applications. In *Molecular Biology in Histopathology*, (2nd edn), Crocker J, Murray PG (eds). John Wiley & Sons Ltd: Chichester, 2003; 1–15.

Chapter in a book

Stevens A, Lowe J. Pathology (2nd edn). Mosby: London, 2000; 224–226.

Entry in a database

Online Mendelian Inheritance in Man [Internet]. Johns Hopkins University. Record No. 191170. Tumour Protein p53; TP53; [Accessed February 14, 2010]: Available from: <http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=191170>

4.21 Tables

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[1] Lang TA, Talerico C, Siontis GC. Documenting clinical and laboratory images in publications: the CLIP principles. *Chest* 2012; **141**: 1626-1632.

[2] Rossner M and Yamada KM. What's in a picture? The temptation of image manipulation. *J Cell Biol* 2004; **166**: 11-15.

[3] Marty GD. Blank-field correction for achieving a uniform white background in brightfield digital photomicrographs. *BioTechniques* 2007; **42**: 716-720.

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Editors. Uniform Requirements for Manuscripts submitted to Biomedical Journals. *Ann Intern Med* 1997; **126**: 36–47. A digital version is available on the [ICMJE web site](#).

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A B C D E F G H I J K L M N O P R S T U V W Y

A

a, atto (10⁻¹⁸)

Å, angstrom unit(s) [10⁻⁸ cm]

aa, amino acid(s)

Ab, antibody

ABC, ATP-binding cassette

AChR, acetylcholine receptor

ACTH, adrenocorticotrophic hormone

AIDS, acquired immunodeficiency syndrome

AMP, adenosine monophosphate (ADP, ATP)

ANOVA, analysis of variance

B

BAC, bacterial artificial chromosome

β-gal, β-galactosidase

BMI, body mass index

bp, base pair(s)

BP, blood pressure

bpm, beat(s) per minute

BrdU, bromodeoxyuridine

BSA, bovine serum albumin
BSE, bovine spongiform encephalopathy
BW, body weight

C

c, centi (10⁻²)
°C, degree(s) Celsius
cAMP, cyclic adenosine monophosphate (cGMP)
CCL, CC chemokine ligand
CCR, CC chemokine receptor
CD, cluster of differentiation
CDK, cyclin-dependent kinases
cDNA, complementary DNA
CDP, cytosine diphosphate
CFC, colony-forming cell
CFTR, cystic fibrosis transmembrane conductance regulator
CFU, colony-forming unit
ChIP, chromatin immunoprecipitation
CHO, Chinese hamster ovary
Ci, curie(s)
CI, confidence interval
cM, centimorgan(s)
CMP, cytidine monophosphate (CDP, CTP)
CMV, cytomegalovirus
CNS, central nervous system
CoA, coenzyme A
COX, cyclooxygenase
cpm, count(s) per minute
CSA, colony-stimulating activity
CSF, colony-stimulating factor
Ct, threshold cycle
CT, computed tomography
CTL, cytotoxic T lymphocyte
CXCL, CXC chemokine ligand
CXCR, CXC chemokine receptor

D

3D, 3-dimensional
d, deci (10⁻¹)
da, deca (10¹)
Da, dalton(s)
DAB, diaminobenzidine
DAPI, 4',6-diamidino-2-phenylindole
DEAE, diethylaminoethyl
DLCBL, diffuse large cell B cell lymphoma
DMEM, Dulbecco's modified Eagle's medium
DMSO, dimethylsulphoxide
DNA, deoxyribonucleic acid
dpm, disintegration(s) per minute
dsDNA, double-stranded DNA
DTT, dithiothreitol

Return to top of Abbreviations

E

E1, E2, E3 etc, embryonic day 1, 2, 3 etc
EAE, experimental autoimmune encephalomyelitis
EBV, Epstein-Barr virus
EC, endothelial cell
ECG, electrocardiogram, electrocardiography

ECL, enhanced chemiluminescence
ECM, extracellular matrix
E. coli, Escherichia coli
ED50, 50% effective dose
EDTA, ethylenediamine tetraacetic acid
EEG, electroencephalogram
EGF, epidermal growth factor
EGFR, EGF receptor
EGTA, ethyleneglycol-bis-(b-aminoethylether)-N,N,N',N'-tetraacetic acid
ELISA, enzyme-linked immunosorbent assay
EM, electron microscopy
EMEM, Eagle's minimal essential medium
EMSA, electrophoretic mobility shift assay
eNOS, endothelial NOS
ER, endoplasmic reticulum
ERK, extracellular signal-regulated kinase
ES, embryonic stem [cell]

F

f, femto (10⁻¹⁵)
F-actin, filamentous actin
FasL, Fas ligand
FBS, fetal bovine serum
Fc, crystallizable fragment [of immunoglobulin molecule]
FCS, fetal calf serum
FDA, Food and Drug Administration
FFA, free fatty acid
FGF, fibroblast growth factor
FISH, fluorescence in situ hybridization
FITC, fluorescein isothiocyanate

G

g, gram
g, unit(s) of gravity
G, giga (10⁹)
GABA, g-aminobutyric acid
GAPDH, glyceraldehyde-3-phosphate dehydrogenase
GC, germinal centre
G-CSF, granulocyte CSF
GFP, green fluorescent protein
GFR, glomerular filtration rate
GLUT, glucose transporter
GM-CFU, granulocyte-macrophage CFU
GM-CSA, granulocyte-macrophage CSA
GM-CSF, granulocyte-macrophage CSF
GMP, guanosine monophosphate (GDP, GTP)
GPCR, G protein-coupled receptor
Gy, gray(s)

H

h, hour(s)
h, hecto (10²)
HA, haemagglutinin
HBSS, Hanks' balanced salt solution
HCV, hepatitis C virus (HAV, HBV, HDV)
HL, Hodgkin's lymphoma
HDL, high-density lipoprotein
H&E, haematoxylin and eosin
HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid
HGF, hepatocyte growth factor

HIF, hypoxia-inducible factor
HIV, human immunodeficiency virus (HIV-1, HIV-2)
HLA, human leukocyte antigen
HMG, 3-hydroxy-3-methyl-glutaryl
HPLC, high-performance liquid chromatography
HPV, human papilloma virus
HRP, horseradish peroxidase
HSA, human serum albumin
HSC, haematopoietic stem cell
hsp, heat shock protein
HUVEC, human umbilical vein endothelial cell

I

IC₅₀, 50% inhibitory concentration
ICAM, intercellular adhesion molecule
ID₅₀, 50% infective dose
IDDM, insulin-dependent diabetes mellitus
IDL, intermediate-density lipoprotein
IFN, interferon
Ig, immunoglobulin (IgE, IgG)
IGF, insulin-like growth factor
I κ B, inhibitor of NF- κ B (I κ B α , I κ B β)
IL, interleukin (IL-12)
i.m., intramuscular(ly)
i.n., intranasal(ly)
iNOS, inducible NOS
i.p., intraperitoneal(ly)
IP, immunoprecipitate, immunoprecipitated, immunoprecipitation
IR, insulin receptor
IU, international unit(s)
i.v., intravenous(ly)

Return to top of Abbreviations

J

JAK, Janus kinase
JNK, c-Jun NH₂-terminal kinase

K

k, kilo (10³)
K, increment on temperature scale Kelvin
kb, kilobase
kcal, kilocalorie(s)
K_d, dissociation constant
kDa, kilodalton(s)
K_i, inhibition constant
K_m, Michaelis-Menten constant
KO, knockout

L

l, litre
LD₅₀, 50% lethal dose
LDL, low-density lipoprotein
LN, lymph node
lod, log odds ratio
LPS, lipopolysaccharide
LUC, luciferase
LV, left ventricle, left ventricular

M

m, milli (10^{-3})
m, metre(s)
M, molar (moles per litre)
M, mega (10^6)
mAb, monoclonal Ab
MALDI, matrix-assisted laser desorption/ionization
MAPK, mitogen-activated protein kinase
Mb, megabase(s)
M-CSF, macrophage CSF
MD, muscular dystrophy
2-ME, 2-mercaptoethanol
MEK, MAPK kinase
mEq, milliequivalent(s)
MFI, mean fluorescence intensity
MHC, major histocompatibility complex
min, minute
miRNA, microRNA
mmHg, millimeter(s) of mercury
MMP, matrix metalloproteinase
mo, month(s)
MOI, multiplicity of infection
mol, mole(s)
MOPS, 3-(N-morpholino)propanesulphonic acid
Mr, relative molecular mass
MRI, magnetic resonance imaging
mRNA, messenger RNA
 μ , micro (10^{-6})
MW, molecular weight

N

n, nano (10^{-9})
n, number in group
N, normal [solution]
N, total sample size
N/A, not applicable
NAD, nicotinamide adenine dinucleotide
NADH, reduced NAD
NADPH, reduced NAD phosphate
ncRNA, non coding RNA
NF- κ B, nuclear factor κ B
NHL, non-Hodgkin lymphoma
NIDDM, non-insulin-dependent diabetes mellitus
NK, natural killer
NKT, natural killer T [cell]
NMDA, N-methyl-D-aspartate
NMR, nuclear magnetic resonance
NO, nitric oxide
NOD, nonobese diabetic
NOR, nonobese resistant
NOS, NO synthase
NP-40, Nonidet P-40
NS, not significant
NSAID, nonsteroidal antiinflammatory drug
NSCLC, non-small cell lung cancer
nt, nucleotide(s)

O

OCT, optimal cutting temperature [compound]
OD, optical density
ORF, open reading frame

Return to top of Abbreviations

P

p, pico (10⁻¹²)
P, phosphate (PO₄)
P, probability
P1, P2, P3, postnatal day 1, 2, 3
PAGE, polyacrylamide gel electrophoresis
PAR, protease-activated receptor
PAS, periodic acid–Schiff
PBL, peripheral blood leukocyte
PBMC, peripheral blood mononuclear cell
PBS, phosphate-buffered saline
PCR, polymerase chain reaction
PDGF, platelet-derived growth factor
PDGFR, PDGF receptor
PE, phycoerythrin
PECAM, platelet–endothelial cell adhesion molecule
PET, positron emission tomography
PFU, plaque-forming unit
PG, prostaglandin
pH, hydrogen ion concentration
pI, isoelectric point
PI3K, phosphatidylinositol-3'-kinase
PIPES, piperazine-N, N'-bis(2-ethanesulphonic acid)
PKC, protein kinase C (PKA, PKB)
PLC, phospholipase C
PMA, phorbol myristate acetate
PMSF, phenylmethylsulphonyl fluoride
PPAR, peroxisome proliferator–activated receptor (PPAR γ)
psi, pound(s) per square inch
PVDF, polyvinylidene difluoride

R

r, correlation coefficient
RA, rheumatoid arthritis
rad, radiation-absorbed dose
RAG, recombination activating gene
RANK, receptor activator of NF- κ B
RANKL, RANK ligand
RANTES, regulated on activation, T cell expressed, and secreted
rbc, red blood cell
RFLP, restriction fragment length polymorphism
RIA, radioimmunoassay
RNA, ribonucleic acid
RNAi, RNA interference
ROS, reactive oxygen species
rpm, revolution(s) per minute
RPMI, Roswell Park Memorial Institute [medium]
rRNA, ribosomal RNA
RT, reverse transcriptase [or reverse transcription in RT-PCR]

S

s, second(s)
SARS, severe acute respiratory syndrome
s.c., subcutaneous
SCF, stem cell factor
SCID, severe combined immunodeficiency disease
SD, standard deviation

SDS, sodium dodecylsulphate
SEM, standard error of the mean
shRNA, short hairpin RNA
siRNA, small, interfering RNA
SIV, simian immunodeficiency virus
SCLC, small cell lung cancer
SLE, systemic lupus erythematosus
SMC, smooth muscle cell
SNP, single nucleotide polymorphism
SOCS, suppressor of cytokine signalling
SOD, superoxide dismutase
SSC, standard saline citrate
STAT, signal transducer and activator of transcription

T

t, time
T, tera (10¹²)
t_{1/2}, half-life
TBS, Tris-buffered saline
TCA, tricarboxylic acid
TCR, T cell receptor
Tg, transgene, transgenic
TGase, transglutaminase
TGCT, testicular germ cell tumours
TGF, transforming growth factor
Th, T helper [cell] (Th1, Th1)
TLC, thin-layer chromatography
TLR, Toll-like receptor
TNF, tumour necrosis factor (TNF- α)
TOF, time of flight
TPA, tissue plasminogen activator
Treg, regulatory T cell
Tris, tris(hydroxymethyl)-aminomethane
TSG, tumour suppressor gene
TRITC, tetraiodoamine isothiocyanate
TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling
TXA, thromboxane A

Return to top of Abbreviations

U

U, unit(s)
UMP, uridine monophosphate (UDP, UTP)
UV, ultraviolet

V

V, volt(s)
VCAM, vascular cell adhesion molecule
VEGF, vascular endothelial growth factor
VEGFR, VEGF receptor
VHL, von Hippel-Lindau disease
VLDL, very low-density lipoprotein
V_{max}, maximum velocity
vol, volume
VSMC, vascular smooth muscle cell
vWF, von Willebrand factor

W

W, watt(s)
wbc, white blood cell

wk, week(s)
wt, weight
WT, wild type

Y
yr, year(s)