ABSTRACT

Breast cancer is the most frequent neoplasm in women and one of the leading cause of death from cancer (Ferlay et al., 2007).

A landmark study (Sjöblom T, et al., 2006) has shown there is still a large number of genes not previously associated to neoplasia that might give us important clues about epithelial tumorigenesis. A systematic analysis of the somatic mutations in breast and colorectal cancers has led to the identification of 189 genes very likely to have been subject to mutational selection during tumorigenesis, that have been termed Candidate Cancer Genes, or CAN genes. The identification of these genes might lead to news avenues of research in basic tumor biology, and have implications at the levels of diagnosis, prognosis and therapeutic targets.

The fraction of mutations that were single base substitutions was similar in breast and colorectal cancers, but the spectrum and nucleotide contexts of the substitutions differed widely between the two tumor types, perhaps reflecting differences in the tumorigenic mechanisms that give rise to them. On average, each breast carcinoma harbored non-synonymous mutations in 52 genes, 9 of which were CAN genes. Among the genes mutated in this tumor type was SIX4, a gene involved in transcription regulation that had not been previously associated to tumor formation.

SIX4 is a member of the SIX family, which belongs to the class II – also called “divergent” – type of homeobox genes and appears to have a modular structure, with different exons coding for different functional regions of the protein. In human SIX4, for instance, exon 1 codes for the Six domain and for a Six-type homeodomain, exon 2 codes for a region of unknown function and exon 3 codes for the transactivation domain.

The purpose of the work reported in this thesis was to help to determine if, indeed, SIX4 plays a role in mammary cancer development. To this end, 48 clinical samples sequence of SIX4 have been screened. No somatic alterations were detected in SIX4, implying that, as for other homeobox genes (Goodman & Scambler, 2001), mutations in SIX4 are not a common event.

A non-synonymous alteration was indeed found in exon 3, in several cases, but comparison to the corresponding normal samples demonstrated it is a hereditary variant. Although this allelic variant had already been described and found to have different frequencies in various populations, it is still plausible that it might contribute to breast
cancer predisposition, since, as noted, exon 3 codes for SIX4’s transactivation domain. It is therefore important to try to determine the functional impact of this variant – and also of the somatic alterations previously detected (Sjöblom T, et al, 2006) – on protein expression levels as well as on transcription activation. We have devised a strategy for functional testing the different SIX4 variants, based on the development of a reporter vector for SIX4 transactivation and vectors for constitutive high-level expression of SIX4 in human mammalian cells.