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GENETIC AND EPIGENETIC ALTERATIONS IN GENOMES

Preservation and regulation of genetic information is essential for proper cell function. Consequently, cells have evolved mechanisms of DNA repair, telomere maintenance, and epigenetic regulation of gene expression patterns. Deregulation of these processes contributes to the appearance and progression of cancer cells, which are characterized by genomic rearrangements and dysregulated gene expression patterns. Studies in our group examine how certain DNA repair processes lead to the insertion of mitochondrial DNA sequences into the nuclear genome. We are also exploring the mechanisms by which tumor cells maintain their telomeres to acquire immortality. We have demonstrated that epigenetic alterations in tumors, involving loss of DNA methylation marks, can lead to the aberrant activation of a particular group of genes. We are currently investigating how epigenetic marks are established on these genes in embryonic cells, and how they become altered in tumor cells.

DNA DAMAGE REPAIR IN FISSION YEAST *SCHIZOSACCHAROMYCES* *POMBE*

S. Lenglez, A. Decottignies

DNA repair processes have been well conserved throughout evolution, and yeast has proven to be a good model for their study. We use *S. pombe* to dissect the mechanisms of DNA double-strand break (DSB) repair, a type of genetic lesion arising after exposure to genotoxic agents or during DNA replication. Chromosomal and extrachromosomal DSBs can be induced experimentally in virtually any kind of cell. Such systems led to the dissection of the two major mechanisms of DNA repair: homologous recombination (HR) and non-

homologous end-joining (NHEJ). In the lab, fission yeast was used to investigate genetic requirements for microhomology-mediated end-joining (MMEJ), a third DNA repair process that was shown to be related to HR (1). From yeast to mammals, different studies reported the insertion of DNA fragments of various sources at experimentally-induced DSBs, including mitochondrial DNA (mtDNA) in budding and fission yeast (2) Interestingly, several studies reported the association of human genetic diseases with *de novo* insertions of mtDNA in the nuclear genome, including a patient exposed to Chernobyl radiations. Moreover, systematic sequencing of eukaryotic nuclear genomes revealed the presence of nuclear sequences of mitochondrial origin (NUMTs) in chromosomes, suggesting that capture of mtDNA fragments at naturally occurring DSBs took place during evolution, remodeling

the nuclear genome. By analyzing fission yeast nuclear genome, we found a strong correlation between NUMT localization and chromosomal DNA replication origins (ORIs). Our data suggest that these mtDNA fragments are not part of the ORI but may have been inserted preferentially next to ORIs because these loci are more prone to breakage (Lenglez et al., Genome Research, in revision).

IMPACT OF TELOMERASE ON TGF- β AND NF- κ B SIGNALING IN HUMAN FIBROBLASTS

M. Mattiussi, G. Tilman, A. Decottignies

Telomeres are specialized protein-DNA structures, which prevent chromosome ends from being recognized as DSBs. Synthesis of telomeric DNA sequences in replicating cells requires telomerase. Cancer cells often show an increased level of telomerase, and this contributes to their unlimited proliferation potential. In some cancers, however, telomeres are maintained in the absence of telomerase activity by one or more mechanisms that are known as alternative lengthening of telomeres (ALT). These two pathways of telomere maintenance are very distinct phenotypically. In telomerase-expressing cells (TEL+), telomere length is very homogenous and telomeres are found at the end of all chromosomes. However, in ALT cells, telomeres are very heterogeneous in length and some chromatids lack telomeres (Fig. 1).

In addition to its role in telomere length maintenance, hTERT has been reported to play non-canonical roles in the cell, including modulation of expression of genes implicated in tumorigenesis, through mechanisms that are still largely unknown. Accordingly, it was suggested that, for a given type of cancer, patients with TEL+ tumors may have a poorer prognosis than patients suffering from ALT tumors. We are interested in understanding how telomerase may impact on gene expression in fibroblasts and, more specifically, how telo-

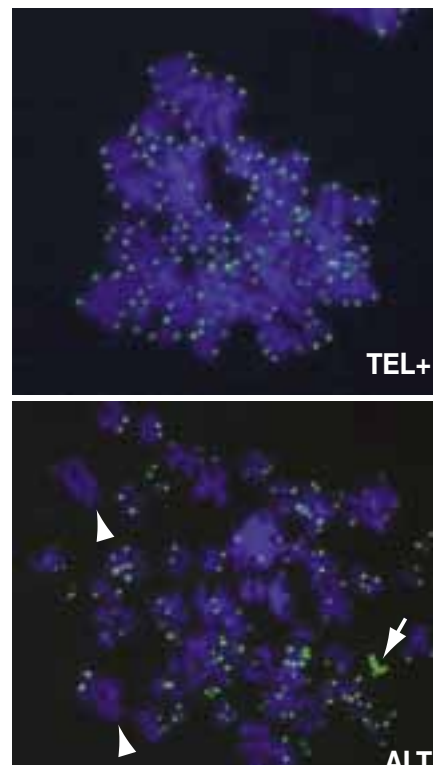


Fig. 1. Telomere-specific fluorescence in situ hybridization (FISH) on metaphase chromosomes of telomerase-positive (TEL+) and ALT cancer cells (ALT). Telomeres are hybridized with a fluorescent telomeric probe (green) and DNA is stained with DAPI (blue). In ALT cells, telomeres are very heterogeneous, and even absent at some chromosome ends (arrowheads). ALT cells are further characterized by the presence of extrachromosomal telomeric DNA (arrow).

merase may modulate TGF- β and/or NF- κ B signaling, two pathways frequently deregulated in tumors. Our data suggest that hTERT may impact on these two signaling pathways through both reduced production of reactive oxygen species and nuclear accumulation of RelA/p65 subunit of NF- κ B.

DNA HYPOMETHYLATION AND ABERRANT GENE ACTIVATION IN CANCER

A. Lorient, G. Parvizzi, C. De Smet

Genomic DNA in multiple species is modified by the addition of a methyl group to cytosines in CpG dinucleotides. This heritable epigenetic modification is associated with transcriptional repression. Cell-type specific DNA methylation patterns are established during embryonic development, and are usually maintained in adult somatic cells.

DNA methylation patterns often become altered in cancer cells. Alterations include hypermethylation of selected promoters, leading to silencing of critical genes such as tumor suppressor genes, and hypomethylation of numerous other DNA sequences. We have shown that genome hypomethylation in tumors results in the activation of a group of germline-specific genes, which use primarily DNA methylation for repression in somatic tissues (4). These genes, which were originally discovered because their activation in tumors leads to the expression of tumor-specific antigens, were named cancer-germline genes. To date, ~50 cancer-germline genes or gene families have been identified. Several of these were isolated in our group (5).

The process leading to hypomethylation of DNA sequences in tumors remains obscure. We undertook to address this issue by using MAGEA1, the founding member of the cancer-germline group of genes, as a model. Detailed methylation analyses of the MAGEA1 genomic locus in expressing tumor cells, revealed preferential hypomethylation within the 5' region of the gene (6). Furthermore, transfection experiments with *in vitro* methylated MAGEA1 constructs, indicated that this site-specific hypomethylation relies on a historical event of DNA demethylation, and on the presence of appropriate transcription factors to protect the region against subsequent

remethylation (6-8). The factors that are responsible for the initial DNA demethylation process and for maintaining cancer-germline gene promoters unmethylated remain to be identified. Genetic screenings with a lentiviral human shRNA library, directed against ~20,000 genes, are currently being performed to identify such factors.

DNA METHYLATION CHANGES ASSOCIATED WITH CELL SENESCENCE AND IMMORTALIZATION

G. Tilman, A. Lorient, C. De Smet, A. Decottignies

In human and mouse cells, recent studies have shown that telomeric and subtelomeric chromatin contains histone modifications that are commonly found in heterochromatin. Increasing evidence also indicates that chromatin modifications at chromosome ends are important regulators of mammalian telomeres. In particular, alterations of either histone modifications in telomeric chromatin or of DNA methylation in subtelomeric regions are associated with telomere length deregulation in mouse cells. In addition, a decreased subtelomeric DNA methylation level in mouse cells was reported to be associated with increased homologous recombination between telomeric sequences (T-SCE for Telomeric Sister Chromatid Exchange), a hallmark of human ALT cells.

This prompted us to evaluate the subtelomeric DNA methylation level of human TEL+ and ALT cancer cell lines (9). We detected a significant hypomethylation of subtelomeric DNA in ALT cancer cell lines when compared to TEL+ cell lines. However, subtelomeric DNA was not hypomethylated in ALT cell lines derived from *in vitro* immortalization of human fibroblasts with SV40 T antigen, although T-SCE frequencies in the latter cells were similar to those in ALT cancer cells (9).

Strikingly, subtelomeric DNA hypomethylation in ALT cancer cells was also associated with lower global DNA methylation. This observation raised the interesting possibility that DNA demethylation in tumor cells may be linked to the process that cells use to escape from senescence and/or crisis, two anti-proliferative barriers thought to require bypass during tumorigenesis. We are currently analysing the histone modifications at telomeres of ALT and TEL+ cells.

We started to address the cellular mechanisms underlying the differences in DNA methylation levels between ALT and TEL+ cancer cells. To this end, we are trying to reproduce, *in vitro*, the demethylation process that operates during tumorigenesis by overexpressing RasV12 oncogene in human dermal fibroblasts. On one hand, RasV12 oncogene is known to induce cellular senescence of primary fibroblasts through activation of the DNA damage response and, on the other hand, this oncogene leads to cellular transformation of p53/pRb-defective cells. As both senescence and transformation may be associated with genomic DNA hypomethylation, we are investigating these two aspects of RasV12 expression. We also wish to compare the DNA methylation profiles of ALT and TEL+ cells that we expect to arise from SV40 T and RasV12-expressing fibroblasts. The experiments are also performed in other cell types, including melanocytes and mammary epithelial cells.

IMPACT OF TELOMERIC TRANSCRIPTS ON HUMAN TELOMERE REPLICATION

N. Arnoult, A. Van Beneden, A. Decottignies

Recent studies indicated that telomeres of eukaryotic cells are frequently transcribed, yielding (UUAGGG)_n non-coding RNAs in mammalian cells, called “TERRAs”. TERRAs have been shown to localize to telomeres where they

may impact on diverse aspects of telomere biology, including telomere replication. Because of the reduced methylation level of subtelomeric DNA in ALT tumor cell lines (9), these cells produce high levels of TERRAs. We are interested in testing whether (and how) TERRAs may affect telomere replication in human cells. During the past few months, we developed the tools to start investigating this.

DNA METHYLATION OF CANCER-GERMLINE GENES IN HUMAN EMBRYONIC STEM CELLS

G. Parvizji, A. Loriot, C. De Smet

As new methylation patterns are established during early embryo development, embryonic stem (ES) cells provide a suitable experimental system for investigating the molecular mechanisms underlying this epigenetic reprogramming process. ES cells possess both DNA demethylation and de novo methylation activities. Each of these opposing activities appears to be targeted to selected DNA sequences. The mechanisms underlying this targeting are still unclear, but likely involve sequence-specific DNA binding proteins and chromatin modifying enzymes. We recently initiated studies on the epigenetic regulation of cancer-germline genes in human ES cells.

We found cancer-germline genes to be repressed and methylated in human ES cells (obtained from Dr. D. Melton, Harvard University, MA), as well as in human embryonal carcinoma (EC) cells, the malignant counterparts of ES cells (10). This indicates that cancer-germline genes are programmed for methylation-mediated silencing in human ES cells. Our data suggest that the repression of cancer-germline genes in EC cells involves the action of the two de novo DNA methyltransferases DNMT3A and DNMT3B, and that local recruitment of these enzymes is associated with specific histone modifications, including underacetylated

histone H3 and unmethylated lysine 4 of the same histone (H3K4). We are currently searching to identify the factors that mediate these histone modifications. Loss of function of such factors may be a prerequisite for demethylation and activation of cancer-germline genes in tumors. Interestingly, we observed that demethylation and activation of MAGEA1 in tumor cells is associated with a marked gain of H3 acetylation and H3K4 methylation.

DEVELOPING PREDICTIVE MARKERS OF RESPONSE TO CHEMOTHERAPY IN BREAST CANCER PATIENTS

F. Fontaine, C. De Smet (BruBreast project: in collaboration with C. Sotiriou and F. Fuks, ULB; J. De Grève, VUB)

Breast cancer is the most frequently encountered type of cancer in women. Although several treatment options are available, one third of the patients eventually die from the disease. The currently used factors for predicting response to therapy are suboptimal and insufficient to explain the differences in survival. The BruBreast project aims to identify markers that would predict the response or resistance to anti-cancer treatment in individual patients with greater accuracy. Practically, the project is accomplished in the context of a multicentric clinical study (coordinated by the Institut Jules Bordet, ULB) aiming at analyzing gene expression profiles associated with response or resistance to epirubicin, one of the most active chemotherapies in breast cancer. We will determine if specific methylation marks are associated with the differentially expressed genes. Our goal is to develop and validate a robust molecular detection kit based on gene expression and methylation markers, which would predict resistance/response to treatment of breast cancer.

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