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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 explore the cellular events leading to genomic instability and the mechanisms by which tumour cells maintain their telomeres to acquire immortality. We have demonstrated that epigenetic alterations in tumours, 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 tumour cells.*

### GENOMIC INSTABILITY IN *SCHIZOSACCHAROMYCES* *POMBE* FISSION YEAST

*S. Lenglez, A. Decottignies*

We use *S. pombe* fission yeast to investigate the cellular events driving genomic instability in eukaryotic cells. To get more insight into the nature of chromosomal fragile sites, we characterized *S. pombe* loci that are naturally prone to double-strand break (DSB) formation. 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 (1). Interestingly, several studies reported the association of human genetic diseases with *de novo* insertions of mtD-

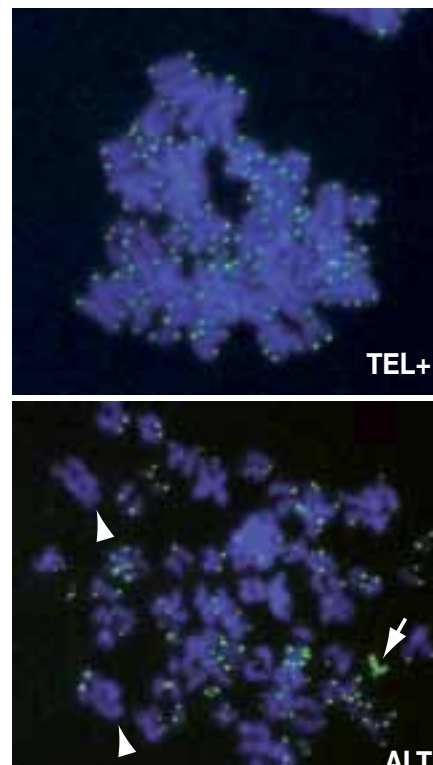
NA in the nuclear genome, including a patient exposed to Chernobyl radiations. Moreover, systematic sequencing of eukaryotic nuclear genomes revealed the presence of nuclear sequences of mitochondrial origin (NUMTs) in chromosomes, suggesting that capture of mtDNA fragments at naturally occurring DSBs took place during evolution and remodelled nuclear genomes. 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 (2). Using an *in vitro* assay, we further showed that subtelomeric loci associated with a replication origin are highly susceptible to DSB formation in conditions of excessive origin firing (2).

## IMPACT OF TELOMERASE ON NF- $\kappa$ B SIGNALING AND CELLULAR RESPONSES TO TNF- $\alpha$ 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 well-established role in telomere synthesis, telomerase exerts non-canonical functions that may promote cancer and stem cell survival, notably as transcriptional cofactor in Wnt- $\beta$ -catenin signaling pathway. The previously reported physical interaction between telomerase and NF- $\kappa$ Bp65 suggested that telomerase may similarly modulate NF- $\kappa$ B pathway, another master regulator of cell proliferation and survival. We investigated telomerase impact on NF- $\kappa$ B signaling in normal human fibroblasts. Strikingly, telomerase overexpression induced constitutive nuclear accumulation of NF- $\kappa$ Bp65 that, however, lacked activating Ser-536 phosphorylation. Although NF- $\kappa$ Bp65 nuclear accumulation constitutively up-regulated IL-6, basal expression levels of most NF- $\kappa$ B target genes were unaffected, arguing against a general hyperactivation of the pathway. Conversely, prolonged culture of telomerase-expressing fibroblasts down-regu-



**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).

lated TNF- $\alpha$  target gene, due to progressive promoter hypermethylation. Telomerase did not either alter NF- $\kappa$ B pathway activation by tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). Interestingly however, owing to its ability to reduce reactive oxygen species (ROS), telomerase impaired *SOD2* transcriptional activation as ROS contribute to TNF- $\alpha$ -dependent *SOD2* induction. Accordingly, other ROS-dependent TNF- $\alpha$ -induced processes, including Mitogen Activated Protein Kinase (MAPK) activation and cell death, were reduced by telomerase. Our results demonstrate a new way by which telomerase, through a reduction of ROS, modulates intracellular signaling and gene expression in response to cytokines (3).

## **DNA HYPOMETHYLATION AND ABERRANT GENE ACTIVATION IN CANCER**

*A. Lorient, 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.

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 (5). 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). The factors that are responsible for the initial DNA demethylation process and for maintaining cancer-germline gene promoters unmethylated remain to be identified.

## **DNA METHYLATION CHANGES ASSOCIATED WITH CELL SENESCENCE AND IMMORTALIZATION**

*G. Tilman, A. Lorient, A. Van Beneden, 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 (7). 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 (7). 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. Indeed, evidence accumulated during the past decade that senescent and cancer cells share similarly altered global epigenetic profiles that includes changes in DNA methylation, is in agreement with the hypothesis that senescence, whether induced by ageing or by oncogene activation, may be a common step in the tumorigenesis process (8). We are currently investigating whether deep DNA hypomethylation may have favoured the emergence of ALT cells during tumorigenesis.

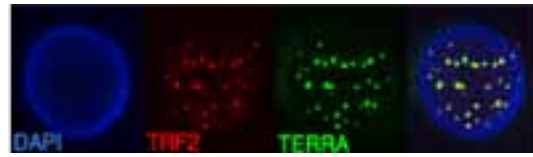
We also started to address the cellular mechanisms underlying the differences in DNA methylation levels between ALT and TEL+ cancer cells. To this end, we are trying to reproduce, *in vitro*, the demethylation process that operates during tumorigenesis by overexpressing RasV12 oncogene in human dermal 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.

## REGULATION OF TELOMERIC TRANSCRIPTS IN HUMAN CELLS

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

Recent studies indicated that telomeres of eukaryotic cells are frequently transcribed, yielding (UUAGGG)<sub>n</sub> 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 regulation of telomerase access to telomeres and DNA damage checkpoint. We are interested in understanding how TERRAs

are regulated in human cells and what their impact is on telomeres, both in TEL+ and ALT cells.



**Figure 3. Detection of TERRA telomeric transcripts by RNA-FISH at human telomeres.** Telomeres are detected by immunofluorescence against TRF2 telomeric protein (red), TERRAs are hybridized with a fluorescent telomeric probe (green) and DNA is stained with DAPI (blue).

## EPIGENETIC REPRESSION OF CANCER-GERMLINE GENES IN HUMAN EMBRYONIC STEM CELLS

*G. Parvizi, A. Lorient, C. De Smet*

The stage at which cancer-germline genes become methylated during human embryo development has not been determined. We found previously that human cancer-germline genes are repressed and methylated in human blastocyst-derived embryonic stem cells and in comparable embryonal carcinoma cells (9). By performing transfection experiments, we now demonstrated that human embryonal carcinoma cells target active de novo methylation towards MAGEA1, as the gene became methylated and silenced following integration into these cells. Consistently, silencing of MAGEA1 in embryonal carcinoma cells depended on the presence of both DNMT3A and DNMT3B de novo DNA methyltransferases. Other chromatin-related silencing mechanisms, namely histone H3K9 and H3K27 methylation, were not associated with MAGEA1 repression in human embryonal carcinoma cells. This distinguishes the human MAGEA1 gene from its murine counterparts, which rely on H3K9

methylation for repression in embryonic cells. Moreover, by analyzing transcription profiling datasets from human preimplantation embryos, we found that transcription of cancer-germline genes increases up to the morula stage, and then decreases dramatically in blastocysts. Altogether our data indicate that human cancer-germline genes are programmed for repression in the blastocyst, and suggest that de novo DNA methylation is a primary event in this process. They also identify species-specific differences in the underlying epigenetic mechanisms. The disparity between mouse and human MAGEA genes is likely attributable to their poor sequence conservation, especially within regulatory regions, and may explain our previous surprising observation that in vitro methylated human MAGEA1 sequences become demethylated following transfection into mouse embryonic stem cells (10).

## UNBIASED SEARCH FOR FACTORS THAT MAINTAIN MAGEA1 METHYLATION

A. Lorient, C. De Smet

We decided to perform an unbiased search for factors that participate in maintaining methylation within the promoter of *MAGEA1*. To this end we have transduced a lentiviral shRNA library into a human melanoma cell line containing a methylated transgene comprising the *MAGEA1* promoter followed by the sequence encoding the green fluorescent protein (GFP). Transduction of shRNAs directed against factors that contribute to methylation maintenance should lead to de-repression of the transgene and emergence of GFP positive clones. GFP positive cell clones, which emerged at different time points after transduction, have been isolated by cell sorting. The shRNA sequences they contain are currently being identified. Single shRNAs directed against candidate genes will be tested individually to confirm activation and demethylation of the *MAGEA1* transgene.

## DEVELOPING PREDICTIVE MARKERS OF RESPONSE TO CHEMOTHERAPY IN BREAST CANCER PATIENTS

V. Rucchin, 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 epirubicine, 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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