Age Brings Wisdom, DNA Methylation, and Sometimes Cancer

The age-dependent DNA methylation of certain stem cell genes may prime cells for malignant transformation, tipping the balance in favor of cancer.

Introduction

It is widely recognized that a number of factors, such as exposure to smoking, chemicals, or steroids, can lead to cancer. One of the mechanisms that may be responsible is an alteration in DNA methylation (DNAm) patterns, resulting in the hypermethylation of tumor suppressor gene promoters. While this represents an important component, it is certainly not the lone trigger of cancer development. Martin Widschwendter, M.D., MRCOG and his team at the University College London's Institute for Women's Health investigated whether DNA methylation of genes targeted by polycomb group proteins (PCGs) in stem cells may also play a role in cancer. PCG target genes (PCGTs) are reversibly silenced in stem cells and become expressed upon differentiation signals. Studying more than 1,000 normal- and disease-state blood, cell, and tissue samples using Illumina's Infinium® HumanMethylation27 BeadChip, they analyzed the methylation status of over 27,000 CpGs—some mapping to PCGTs. From these data, they identified a distinct PCGT CpG hypermethylation pattern that exhibited a linear increase with age, was ubiquitous across tissue types, and was significantly elevated in cancer tissue samples[1]. The results could spark development of a new generation of diagnostics and therapeutics to combat cancer, a disease projected to cause 12 million deaths worldwide in 2030[2].

Stem Cells Caught in a Holding Pattern

Stem cells maintain their pluripotency through the reversible repression of PCGTs. The PCG complex is a protein complex comprising at least three components–SUZ12, EED, and EZH2—that binds to PCGTs and prevents these genes from being transcribed. Micro-environmental triggers, such as paracrine or endocrine signals, release the protein complex, reversing repression of PCGTs and enabling the stem cells to differentiate. Over the last few years, Dr. Widschwendter's team and others have demonstrated that stem cell PCGTs are far more likely to be methylated in cancer, suggesting a stem-cell origin model for cancer[3–5]. It is thought that PCGT methylation locks the stem cells into a perpetual state of self renewal, predisposing them to subsequent malignant transformation.

With age being the most important risk factor for cancer, Dr. Widschwendter and his team hypothesized that its carcinogenic potential may be conferred by PCGT methylation that irreversibly stabilizes stem cell features. To identify age-dependent CpGs that might be important in the biology of epithelial cancers, they decided to look for an age-dependent methylation signature in peripheral blood cells, validate that signature in independent blood samples and normal epithelial tissues, and then test the biological relevance of this signature in epithelial neoplasias. The HumanMethylation27 BeadChip was chosen "because it was the most efficient and cost-effective choice to perform high-throughput methylation studies looking at a large number of CpGs," according to Dr. Widschwendter. "It had been validated against a variety of other technologies, including MethyLight, which we were using at the time."

Dr. Martin Widschwendter, M.D., MRCOG is a Professor in Women's Cancer and a Consultant Gynecological Oncology Surgeon at the University College London.

Dr. Widschwendter's team began their studies testing 261 blood samples, 148 from healthy women and 113 from ovarian cancer cases. Using a linear statistical model and adjusting for confounding factors, Andrew Teschendorff, Ph.D. (the lead statistician on Dr. Widschwendter's team) noticed that the DNAm signature was very similar in all samples regardless of type or disease state.

"We found that 226 CpGs were hypermethylated with age, while 363 CpGs were hypomethylated," Dr. Widschwendter said. "From this group of 589, we derived a core methylation signature consisting of 69 CpGs mapping to 64 unique PCGT loci. There was a five- to six-fold over-representation of PCGTs with age-driven CpG methylation. The average methylation profile of these CpGs increased monotonically over an age range spanning greater than 25 years (50–80 years). In contrast, only 11 PCGTs were hypomethylated with age."

The expression levels of the 64 PCGT genes were measured in normal, cervical cancer, and ovarian cancer tissues and found to be significantly lower in the cancer tissues. This supported the role of methylation in suppressing these genes and implicated their functional relevance in cancer.

"Interplay between epigenetic and non-genetic factors has been postulated before, but here we have provided visibility into what could actually be happening," Dr. Widschwendter said. "In this case, PCGT methylation limits cells' potential for plasticity, leaving them in a less differentiated state, and priming them to become cancer cells. When additional genetic alterations occur, such as p53 mutations, the cells become cancerous."

Methylation Levels Increase in Disease-State Tissues

To determine the pervasiveness of the methylation signature, over 500 samples from both sexes were analyzed, including blood, mesenchymal stem cells, and tissue samples from healthy individuals and patients with Type 1 diabetes and ovarian cancer. While the magnitude of the methylation changes varied between studies and tissues, the PCGT signature exhibited the same, age-related DNAm changes independent of sex, disease state, tissue, and cell type. "We were very surprised by the consistency of the PCGT age-driven methylation signature," Dr. Widschwendter said. "This signature has now been validated using several different technologies[6–7]."

To determine the difference in methylation signatures between normal and disease state tissue, the team performed DNAm profiling of age-matched cervical smear samples from premenopausal women with normal smears and with smears exhibiting dysplasia. The PCGT CpGs in the dysplasia samples were uniformly more heavily methylated than those in the normal samples.

"The age-PCGT CpG methylation signature was able to discriminate the dysplastic samples from the non-dysplastic samples better than any of the other 10,000 random combinations of PCGT or non-PCGT signatures," said Dr. Widschwendter.

Foundation for Future Diagnostics

While this discovery could be applicable to all cancers, the team is more focused on women's cancers, such as ovarian, endometrial, cervical, and breast. They are working with a group at the University of Manchester led by Professor Henry Kitchener in the School of Cancer and Imaging Sciences which has collected liquid-based cervical cytology samples from 25,000 individuals to assess the ability to use the PCGT age-related methylation signature as a diagnostic tool for cervical cancer. "Today, technicians spend eight to ten hours in front of a microscope assessing cervical cytology samples–a very laborious, time intensive, and mistake prone procedure," Dr. Widschwendter said. "A faster, more accurate molecular diagnostic assay to replace morphology would be a huge advantage in diagnosing cervical cancer."

Additional research is underway to test the signature's ability to identify ovarian and breast cancers. Principal investigators Ian Jacobs and Usha Menon at the Institute for Women's Health, which houses Dr. Widschwendter's department, are running the largest prospectively randomized clinical trial in the world, recruiting 200,000 women over the last 10 years for ovarian cancer screening. "Since the samples were collected, about 500 of these women have developed ovarian cancer and more than 4,000 have developed breast cancer," Dr. Widschwendter said. "We are using the HumanMethylation27 BeadChip to analyze DNAm in these samples and are looking forward to publishing the results."

Potential as a Cancer Prognostic

It may be that these DNA methylation patterns are surrogates for life-long exposure to carcinogenic influences. Dr. Widschwendter and his team are studying whether this signature could be used to predict the risk of ovarian and breast cancer. The Müllerian duct epithelium, which forms the fallopian tube and the endometrium, has been identified as the cell of origin for at least some forms of ovarian cancer. His team has identified PCGTs HOXA9 and HOXA11 as being heavily methylated in the Müllerian duct epithelium cells of women with ovarian cancer, but not in those from age-matched women without ovarian cancer[8].

"To identify risk, we have realized we need to assay purified cells from specific compartments, rather than whole blood samples," Dr. Widschwendter said. "We are looking forward to using Illumina's new HumanMethylation450 BeadChip to analyze these endometrial cells for epigenetic alterations that may indicate ovarian cancer predisposition. This BeadChip will enable the analysis of a large number of samples and provide a global epigenomic overview of what is occurring."

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Available in late 2010, the HumanMethylation450 BeadChip will provide >450,000 sites of expert-selected coverage at high throughput and a low price. It is an ideal choice to support genome-wide methylation analysis across large sample populations, such as the studies that the team will soon begin in breast cancer. One of the triggers of breast cancer is life-long exposure to estrogen and progesterone. In earlier research performed by the team, it was demonstrated that genes which are estrogen receptor targets are less methylated in women with breast cancer than in women who are cancer-free[9]. "We will be studying vaginal epithelial cells, which are very hormone sensitive, to determine if there is an epigenetic profile resulting from lifelong hormone exposure that could act as a surrogate for breast cancer predisposition. It may seem like a roundabout way of attacking the problem, but it could be a revolutionary diagnostic approach. To test this approach, 2,000 to 3,000 cases and controls will need to be analyzed."

Confirming the Signature's Functional Importance in Cancer

Developing therapeutics based on this discovery will require further validation of the biological relevancy of the age-PCGT methylation signature. Typically, this would involve re-expression of the genes in question. However, current technology does not enable the re-expression of a well-defined set of genes such as those in this signature. Beyond performing gene expression studies, the team has turned to other means to confirm functional importance of the PCGT methylation signature in cancer.

"A recent paper in the journal Stem Cells reported that when pluripotency was induced in normal cells, these 69 PCGTs were dramatically demethylated[10]," Dr. Widschwendter said. "Inducing pluripotency could provide an interesting way to look and compare the phenotype of a reprogrammed cancer cell, in particular the function of these 69 PCGTs. We are just beginning work in that area."

Casting a Wider Net in Studying PCGT CpGs

The discovery of the PCGT age-related methylation signature has spawned a number of new research studies. "New technology, such as the HumanMethylation450 BeadChip, will enable us to look at a far larger number of PCGTs," according to Dr. Widschwendter. "By studying CpGs across the entire genome, we will be able to dissect differences in expression and their role in cancer, forming the basis of new diagnostics and therapeutics."

Is it possible that these methylation patterns are present in younger patients with aggressive forms of cancer? "It is very possible, which makes this research that much more exciting," said Dr. Widschwendter. "While age is by far the strongest demographic risk factor for cancer, it may be this methylation signature that is the trigger for cancer development."

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