ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Edith HEARD
Professeur au Collège de France

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ENSEIGNEMENT

COURS – *EPIGENETICS AND CANCER* (ÉPIGÉNÉTIQUE ET CANCER)

Introduction

In this series of lectures, cancer is explored from an epigenetics perspective. Today, cancer is increasingly recognized as having both a genetic and an epigenetic basis. Although in the latter half of the twentieth century the genetic view of cancer prevailed, thanks to the discovery of oncogenes and tumor suppressors, we now know that epigenetic changes, whereby changes in gene expression occur that are not due to underlying DNA sequence changes, can be as important. During embryogenesis, epigenetic processes are implicated in perpetuating stable gene expression patterns to preserve cell identity and function. In cancer, such processes maybe disrupted, with the rewiring of gene expression and signaling pathways that can result in uncontrolled cell proliferation, change or even loss of cell identity, and ultimately to invasion and metastasis. Since Laennec, parallels had been noted between cancer and development (epigenesis) with the idea that cancer might consist of the inappropriate acquisition of properties of cells at different developmental stages. Indeed, some of the earliest described cancers were teratomas (from the Greek words “teras” (monster) and “onkoma” (swelling) coined by Virchow in 1863), which can have anatomically identifiable features, such as fingers, teeth and

hair. Although the molecular pathways may be similar in cancer and development, the "rules" of their use are very different. Indeed, unlike embryogenesis where the same genome gives rise to many different epigenomes, in cancer cells both genomes and epigenomes change. In fact, tumors are evolutionary entities, with both genetic and epigenetic changes occurring, that can enable rapid selection. Recent new insights thanks to the sequencing of human cancer genomes, as well as genetic manipulation in model organisms, have reconciled these two views and led to the exciting discovery that many of the mutations in cancer actually lie in genes encoding proteins and non-coding RNAs involved in epigenetic processes. Thus mutations in epigenetic modifiers, such as DNA methyltransferases or demethylases, chromatin enzymes and remodelers can promote oncogenesis, by altering epigenetic marks that impact on gene expression and genome stability. Thanks to functional studies in mouse models, our understanding of specific epigenetic mechanisms and their roles in cancer has increased dramatically. Furthermore, it is now realized that epigenetic plasticity likely plays a key role in generating cellular heterogeneity within tumors, and enabling the dialogue with the stroma that can facilitate cell proliferation, angiogenesis as well as invasion. Today, we understand that the interplay between genetic and epigenetic changes in cancer is implicated at every step of tumor progression, from the incipient neoplasm, through to metastasis.

1. *A brief history of cancer and epigenetics* (Une brève histoire du cancer : génétique et épigénétique)

In the first lecture, a historical overview of cancer was provided, from pre-historic times to the present day. Cancer is not just a disease of modern times but has afflicted humans over the ages and all over the world. Tumor masses have been found in fossilized bones of dinosaurs and humans from pre-historic times and cancerous growths were detected in Egyptian and Peruvian mummies dating back to 1500 BC. Hippocrates first coined the term cancer, when he noted that the forms that blood vessels that feed a tumor resemble the claws of a crab. However, it was only in the past few centuries that pathologists actually recognized the nature of cancer and tried to define it and understand it's basis.

Virchow was one of the first to define cancer as uncontrolled cell growth. He described and named leukemia in the 1840s and defined cancer, using microscopy on specimens from autopsies. In 1855, he proposed that cancers arise by *activation* of dormant cells due to severe chronic irritation. Following on from his seminal proposition that all cells come from cells (*Omnis cellula e cellula*) he went on to define cancer as a disease involving uncontrolled cell growth. However he did not really propose a mechanism for how this begins.

Boveri was the first to suggest a role for abnormal chromosomes in cancer. Thanks to his studies of sea urchin development, where he found that only sea urchin embryos possessing the full set of 36 chromosomes could develop normally, Boveri proposed a theory for cancer in his 1902 monograph. This was based on four main tenets: first, he pointed out that cancer is a cellular problem; second, that cancers originate from a single cell; third, that this cell has an abnormality of its chromosomal constitution; and fourth that the chromosomal abnormality which is passed on to all the descendants of the cell of origin is the cause of rapid cell proliferation. He made several visionary predictions—including the existence of oncogenes and tumor suppressor genes—of cell cycle checkpoints—of loss of cell adhesion in metastasis—

of the sensitivity of malignant cells to radiation. His theory met with tremendous criticism at the time and was only resurrected much later. The discovery of the Philadelphia translocation that leads to high frequency leukemia vindicated Boveri's theory. However chromosomal abnormalities are far less frequent than alterations in gene. The notion that came later, that cancer can be induced by exogenous chemicals and by radiation, added support to the genetic basis of cancer.

Numerous other theories have been proposed over the years. In addition to being a problem of cell proliferation and chromosomal alterations, cancer has been proposed to be an epigenetic process and to be linked to problems in cellular differentiation and tissue organization. Indeed, tumor growth and "morphogenesis" (however disorganised) can be considered as a form of "epigenesis": ie growing complexity from a single cell, or clone of cells, to a complex "organism". We now know that the same molecules and signaling pathways are exploited in cancer. However, in cancer, in addition to the changing phenotype there is also a changing genotype. PC Nowell (1976) proposed that cancers evolve through branched evolutionary trajectories fuelled by genomic changes—as predicted by Boveri. This could be akin to a Darwinian process, whereby a tumor is an ecosystem and cells fight to survive and proliferate.

Perhaps the theory that propelled cancer into its molecular era was the tumor virus theory, proposed by Rous in 1911 (for which he obtained the Nobel prize only >50 years later, in 1966). He observed that a malignant tumor (a sarcoma) growing on a domestic chicken could be transferred to another fowl simply by exposing the healthy bird to a cell-free filtrate. Thus, cancer could be virally transmitted (Rous sarcoma virus, retrovirus). This led to a new field of tumor virology and the discovery of further tumor viruses. Importantly it laid the foundations of molecular mechanisms of carcinogenesis. The work on RNA tumor viruses such as the Rous sarcoma virus, led to many discoveries in the 1970s and 1980s, including that of reverse transcriptase, a watershed event in molecular biology, providing the means for generating cDNA and the key to reverse transcription–PCR (RT-PCR). Work on tumor viruses also led to the discovery of oncogenes and proto-oncogenes, such as Src (thanks to Rous sarcoma virus), which normally serves to activate cell division when the cell receives an appropriate signal; while the mutant form (oncogene) causes unrestrained activation of cell division. Many oncogenes have since been discovered—with roles in preventing cell cycle inhibition, over-stimulating the cell cycle, avoiding cell death (apoptosis), preventing cell contact inhibition, altering metabolism and promoting invasion. A few years later, another category of genes which when mutated cause cancer predisposition were discovered: tumor suppressors. Based on studies of familial, hereditary cancers, mutations in the first tumor suppressors such as retinoblastoma genes were discovered and it was found to be involved in regulating the cell cycle. Tumor suppressors (TS) were quickly shown to function in many key cellular processes including the regulation of transcription, DNA repair, cell-cell communication. Loss of function of these genes leads to abnormal cellular behavior, though their roles in vivo were less easy to define. Nevertheless, the accumulating evidence suggested that most cancers showed alterations in one or more TS and oncogenes. In normal cells, these two groups of proteins work together to regulate cell division but in cancer cells the controls are no longer functioning properly. In other words, oncogenes drive cancer while tumor suppressors prevent it.

In summary, this era of cancer research from the 1970s onwards, led to the discovery of many of the genes involved in cancer, and helped to uncover their roles not only in cancer, but in normal cells. Thanks to the compelling evidence that

mutations in genes could trigger oncogenesis, the view that cancer was largely genetic thus prevailed. As Bishop (Nobel Prize in 1989) stated: "we proved that cancer always arises from our own genes, and that damage to those genes is fundamental to the growth of cancer cells… all cancer is genetic, although that doesn't mean that cancer is always inherited." The typical roadmap for cancer proposed was that a series of mutations in a cell would cause it to proliferate more than its immediate neighbors. As the cluster of dividing cells grows over time, further mutations turn atypical hyperplasia into a cancer (carcinoma). The spreading of cancer cells to other tissues and organs (metastasis) occurs when the adhesion of these cancerous cells breaks down, and they are able to travel easily to new locations.

However, it was recognized even at the time that this view might be too simple. First, different cancers seem to involve very different sets of genes (except for specific hematological cancers). Second, somatic mutation rates do not easily explain the rapid evolution of many tumors. Third, this view did not adequately explain the many chromosomal aberrations typical of cancer cells. Fourth, it failed to explain the genetic diversity among cells within a single tumor and finally it did not explain frequent resistance to therapies. As Robert Weinberg put it "Each tumor seemed a unique experiment of nature—acquiring a unique set of mutant genes in an unpredictable chronological order…" (Weinberg, *Cell*, 2014). Alternative models were therefore required. For example, a role for master genes controlling cell division, that might lead to abnormal replication of chromosomes, causing whole sections of chromosomes to be missing or duplicated, with changes in gene dosage contributing to cancer. Chromosomal catastrophic events ("big leaps") were also evoked. Finally epigenetic alterations at specific genes (epimutations) and/or global epigenetic changes were also proposed. In particular, Holliday proposed in 1979 an epigenetic theory of carcinogenesis, whereby heritable changes in gene activity could be due to DNA methylation. He proposed that DNA methylation could act either by "shutting off" one or both alleles—or by inducing mutation. In particular, global DNA hypomethylation could lead to mutations—such as chromosome rearrangements. Epigenetic changes could thus explain many aspects of tumor formation and progression. Indeed, most genetic changes lead to all or nothing gene expression changes, whereas epigenetic changes can lead to range of expression levels and this could help explain tumor heterogeneity. Dosage also appears to be a key aspect for some proteins in helping to provide a cellular selective advantage in cancer—thus epigenetic silencing or activation of some genes might be important. Nevertheless, the fact that much of the epigenetic data generated was correlative led to much skepticism, particularly from vociferous proponents of the genetic basis of cancer.

It was in this context, that the advent of whole genome sequencing, as well as powerful genetic engineering approaches, brought timely evidence in support of alternative models and new insights into the genetic and epigenetic nature of cancer.

2. *Cancer genomes and epigenomics: From maps to mechanisms* (La génomique et l'épigénomique des cancers : de la description aux mécanismes)

The prevailing view put forward by Bert Vogelstein and others, was that mutations in genes for tumor suppressors and oncogenes lead to cancer and that it is mainly a genetic disease caused by an accumulation of mutations in genes that control the birth, growth, and death of the body's cells. A cell must acquire multiple mutations

before it becomes cancerous. However, cancer is a complex condition and tumors are dynamic "ecosystems", with evolving genotypes/phenotypes as well as interactions between different cancer cells, the stroma, the immune system, and even bacteria. Thus the emerging realization is that cancer cells seems to have inherent plasticity and evolvability, both hallmarks of epigenetics. Hence the increasing interest in epigenetics and epigenomics. The frequent global loss of 5-methyl cytosine (5mC) in some cancers, as well as the *CpG island methylator phenotype* (CIMP) (Toyota *et al.*, 1999), and global or regional changes in chromatin structure/state led to increasing interest in the possible role that epigenetic changes might play. However, the challenge in cancer was that interpretation of epigenomic changes is difficult without the matching genomic information, because unlike in normal cells, in cancer there are multiple "genomes" and "epigenomes". The advent of high throughput sequencing of human tumor genomes and epigenomes and the concerted international efforts to sequence multiple different types of cancer, has been transformative.

Many lessons are being learned thanks to these tumor genomes. For example, mutation rates are much more variable than expected: from <0.1/Mb to ~100/Mb (in mutagen induced tumors eg lung cancer (tobacco smoke), melanoma (UV). Also a wide array of mutational patterns is found both across and within tumor types. This might be due to extrinsic factors (UV, tobacco) or intrinsic patterns eg DNA repair defects (MLH/MSH mutations in colorectal and other cancers). Another important result was the frequency and type of chromosomal gains and losses. A surprise was the discovery of catastrophic phenomena (chromosomal shattering (chromothripsis), producing tens/hundreds of rearrangement affecting just one or a few chromosomes in different tumor types. This information as well as the capacity to sequence different regions of a tumor, and more recent single cell approaches, has provided insights into the nature of tumor evolution. Thus, rather than the gradual appearance of mutations and natural selection (Darwinian model), massive events such as chromothripsis can generate several genomic lesions in one "big leap" with potential to drive cancer (macro-evolution). This can lead to so-called "Hopeful Monsters"—chromosomal rearrangements that usually lead to death but occasionally give rise to something "greater" (Goldsmith).

Whole genome sequencing of tumors also revealed many new candidate genes that might "drive" tumorigenesis, involved in signalling pathways that regulate 3 core processes: cell fate, cell survival and genome maintenance. However, some cancers had no/few mutations within any known cancer genes but an increasing number of non-coding sequence mutations have been found, that have potential aberrant activation and silencing of cancer genes. Indeed, the recent advent of chromosome conformation capture techniques has enabled researches to uncover potential long range regulatory elements of genes. Subtle mutations affecting regulatory or chromosome structural elements, sometimes at very long distance (100s kilobases) may be sufficient to activate "oncogenes" or inactivate tumor suppressors.

Perhaps the biggest surprise from the tumor genome sequencing projects was the discovery that many novel cancer genes are involved in chromatin functions. For example, several group have reported thathistone H3.3 Lys 27-to-methionine (K27M) mutation in one of two alleles leads to very specific gliomas. This mutation reprograms epigenetic landscapes and gene expression, with genome wide loss in H3K27me3 but specific aberrant enrichment at several hundred genes, which may drive tumorigenesis. Many other mutations were found for genes encoding chromatin remodeling proteins, histone modifiers and DNA methyltransferases/demethylases.

This surprising result suggests that altered epigenetic machinery can indeed lead to altered gene expression, genome stability and cellular phenotypes in a cancer context.

3. *Epigenetic control of genes and genomes in cancer* (Contrôle épigénétique des gènes et des génomes dans le cancer)

In this lecture, the potential roles of epigenetic changes in cancer were discussed, particularly in light of the recent genomic data. The questions dealt with included the following: are epigenetic changes simply a consequence of gene expression changes due to DNA sequence mutations and genomic instability in cancer? Or might epigenetic changes *contribute* to cancer, by causing stable (potentially reversible) alterations in gene expression? (in the soma or even in the germ line?); Can epigenetic changes induce mutations in cancer—cytosine deamination, or loss of repetitive element control, or aberrant silencing of DNA repair genes; Can epigenetic changes contribute to tumor cell heterogeneity, and to the plasticity underlying phenotypic changes eg during invasion or metastasis; How can a global knowledge of the epigenetic characteristics of cancer cells be used for translational purposes (diagnostic, prognostic, therapeutic…)? Epigenetic changes allow gene expression patterns to be reprogrammed. This can lead to changes in cell identity, cell behavior (invasion, migration), generating cell diversity. Transposable elements are key targets for epigenetic control. The loss of this control can induce aberrant nearby gene expression, as well as mutations, and impact on DNA repair. Such repeat mobility can activate oncogenes, silence tumor suppressors. I summarise the genomic and epigenomic data pointing to a role for mutations in epigenetic modifiers epigenetic changes, such as DNA methylation. DNA methylation represents a key control mechanisms for the repression of repetitive elements. Mobile DNA elements can restructure cancer genomes and thus it can be expected that epigenetic changes would impact on their expression and/or mobility. This is a rapidly evolving field and will be covered in future lecture series.

The types of epigenetically altered genes that might be implicated in cancer include acquired epimutations—proposed to occur either in addition to a mutation in a TS, in tumors associated with familial cancer syndromes cause by heterozygous germline mutations; or else as aberrantly activated oncogenes; and constitutional epimutations—that already present (and widespread) in somatic cells, prior to disease onset. In addition, epimutations can be classified either primary—where the epigenetic change is induced in parental germ line or early embryo; or secondary—where the epimutation is a consequence of DNA sequence polymorphism/mutation. Constitutional epimutations provide an alternative mechanism to genetic mutation for cancer predisposition. In cancer-affected families, one can sometimes see inter-generational inheritance of constitutional epimutation. This can be primary (ie non-DNA sequence based) or secondary (ie do to DNA seq variant). Overall, although there is some evidence for constitutional epimutations; most of them are due to the secondary class ie there is an underlying DNA sequence change that drives the altered epigenetic state.

Finally the potential mechanisms underlying epigenetic instability in cancer are discussed. Replication stress can lead to loss of silent or active memory states if chromatin is not properly replicated for example at stalle. Metabolic stress can also have dramatic effects on chromatin as various metabolites impact on epigenetic modifiers. Oxidative damage induces formation and relocalization of a silencing complex that may explain cancer-specific aberrant DNA methylation and transcriptional silencing. During

ageing, epigenetic drift has been shown to occur. However, the discovery of many mutations in epigenetic modifiers in several types of tumors, offers a likely explanation for many of the more regional or genome-wide changes observed in cancer and opens up a new era of exciting research in cancer epigenetics.

4. *Epigenetic pathways in cancer (part 1)* (Voies épigénétiques du cancer [I])

In this lecture, the mutations that have been identified in DNA methylation enzymes in cancer was discussed. DNA methylation is one of the best known epigenetic marks. The enzymes that apply this modification to DNA include de novo methyltransferases, DNMT3A and 3B; and maintenance methyltransferases, DNMT1. In addition DNA methylation can be lost by the conversion to 5-hydroxy methylation via the TET enzymes. Mutations in DNA methylation machinery occur at high frequency in hematopoietic malignancies eg DNMT3A mutations are found in: AML (30%); Myeloproliferative neoplasia (MPN) (7–15%); Myelodysplastic syndrome (MDS) (8%); TET2 is frequently mutated in myeloid disease: AML (7–23%), Chronic myelomonocytic leukemia (CMML) (50%), MDS (10–20%); IDH1/2 mutations found in: AML (16-19%), MPN (2-9%), MDS (3%). the TET enzymes require a-ketoglutarate for their activity and are inhibited by the 2HG oncometabolite product of mutant IDH1/2. TET2 and IDH1/2 mutations thus act, at least in part, through a common mechanism. As would be expected, these mutations rarely co-occur in AML. Interestingly, however, TET2 and DNMT3A mutations frequently co-occur in MDS, pointing to an as-yet unexplained cooperativity between dysregulation of 5mC and 5hmC in leukemogenesis.

Mechanistic and functional tests that have been used to support the potential implications of these DNA methylation modifiers is accumulating. Both over expression and under expression of DNMT3A and B have been shown to result increased tumor frequencies in mouse models. Loss of Dnmt3b accelerates lymphoid tumor development in Dnmt3a-/- mice. Mutations in DNMT3A frequently found in human myeloid and lymphoid malignancies. Allelic losses reported in 48% non-Hodgkin lymphomas. Long-term DNMT3A inactivation in mice leads to impaired differentiation of hematopoietic stem cells (HSCs) resulting in accumulation of undifferentiated cells. DNMT3A loss may promote tumorigenesis in multiple hematopoietic lineages. DNMT3B on the other hand is rarely mutated in human hematologic malignancies. Mouse models for Dnmt3a mutations reveal that HSC self-renewal promoting genes are normally silenced by Dnmt3a upon differentiation. Loss of Dnmt3a function appears to promote a progressive expansion of long term HSCs probably due to inability to adequately repress genes involved in self renewal.

In the case of the TET enzymes, TET1 was initially identified through fusion to MLL (KMT2A) in patients with acute myeloid leukaemia. Importantly, the TET1-fusion may have lost its 5mC oxidase activity but recruit unknown factors aberrantly targeted to MLL genes. *TET2* mutations were since demonstrated to be one of most frequent lesions in myeloid lineage malignancies. Importantly, these myeloid-lineage conditions are susceptible to therapy aimed at inhibiting DNA methylation. Mouse models have shown that Tet2 is a crucial regulator of self-renewal and differentiation in HSCs, supporting a role for Tet2 in normal haematopoiesis. Downregulation of TET expression also seen in human breast, liver, lung, pancreatic and prostate cancers. Acute loss of TET activity in mouse models leads to aggressive myeloid cancer. Tet2/

Tet3 are both highly expressed in mouse HSCs: deletion of either leads to aberrant hematopoiesis (enhanced self renewal, preferential differentiation to myeloid lineage). Acute elimination leads to rapid development of aggressive, fully-penetrant and cell-autonomous myeloid leukaemia. This is preceded by aberrant differentiation of HSC/progenitor cells, impaired erythroid and lymphoid differentiation and strong skewing to the myeloid lineage. In these mice a progressive accumulation of phospho-H2AX and strong impairment of DNA damage repair pathways is observed suggesting a key role for TET proteins in maintaining genome integrity. Intriguingly, the aberrant methylomes associated with Tet mutant induced leukemia not easily connected to changes in gene expression. Thus the precise mechanism that leads to cancer when the TET proteins are mutated is still very much an open question and could be both at the level of aberrant gene expression and/or at the level of genome instability.

Finally, in the case of the IDH1/2 enzymes: IDH1 and IDH2 genes encode isocitrate dehydrogenases. IDH1/2 mutations inhibit Tet2 (and other enzymes) and affect DNA methylation patterns. IDH1/2 mutations are frequently found in human glioblastomas and cytogenetically normal acute myeloid leukaemias (AML). Gain-of-function mutations drive the synthesis of the 'oncometabolite' R-2-hydroxyglutarate (2HG) instead of a-ketoglutarate (aKG). *IDH1/2* mutations are associated with a specific DNA hypermethylation profile in AML. Expression of mutant IDH1/2 induces an increase in global 5-methylcytosine levels and IDH1/2 mutations inhibit the hydroxylation reaction of methylcytosine by TET2. Importantly, expression of IDH2 mutants or loss of TET2 both impair myeloid differentiation, with increased stem/progenitor cell marker expression, suggesting that they have shared proleukemogenic effects. A mouse model of human AML in which an IDH1 single amino acid change was introduced, induced a leukaemic DNA methylation signature. Mutants show increased early haematopoietic progenitors, develop splenomegaly and anaemia with extramedullary haematopoiesis and a dysfunctional bone marrow niche. In another recent study, IDH mutations were found to promote tumor formation (gliomas) by disrupting chromosomal topology and allowing aberrant regulatory interactions that induce oncogene expression. Importantly, mutant IDH1/2 proteins are the targets of emerging drug discovery effort.

In summary, mutations in DNA Methylation enzymes (DNMT3A, TET1/2 and IDH1/2) are frequent in some cancers (such as leukemia and lymphoma). Dynamic DNA methylation patterns in coding and non-coding regions are found during hematopoietic transformation (tumor formation). Intriguingly similar phenotypes are found in Dnmt3a KO and Tet2/3 KO mice (ie increased HSC self renewal, myeloid skewing and transformation), yet loss of Dnmt3a should lead to decreased 5mC, while loss of Tet enzymes should lead to increased 5mC. However, the effects in all cases may be due to the decrease in 5hmC products. The roles of DNA Methylation enzymes and of 5hmC in cancer are still not clear and could be at the level of gene expression and genome stability. Whatever its functions, aberrant DNA methylation can define leukemia and lymphoma subtypes. It is thus of powerful prognostic value and a therapeutic target.

5. *Epigenetic pathways in cancer (part 2)* (Voies épigénétiques du cancer [II])

In this lecture, the roles in cancer of chromatin complexes such as Polycomb and Trithorax was covered. Active and inactive states of genes expression established by

transcription factors are maintained during cellular differentiation by Polycomb (PcG) and trithorax (trxG) over multiple cell divisions. Altered TrX and PRC activities are found in cancer via fusions proteins (eg MLL) or mutations in some of these proteins or in their targets (eg histones, such as H3K27me3). Solid tumors show possibly neomorphic histone K27 mutations (mimicking H3K27me2), UTX mutation, EZH2 amplification, and/or overexpression due to genomic loss of the repressive microRNA miR101, as well as amplification/overexpression of the PRC1 member BMI1, and ymphoma exhibits gain-of-function mutations of EZH2, which is consistent with a gain of Polycomb repression. In myeloid malignancies and ALL, particularly early T cell precursor ALL, show mutations that may sabotage Polycomb repression. Given the vast number of tumor types that these complexes might have a role in, I chose to focus on paediatric and adult glioblastomas. Glioblastomas are the most frequent and aggressive malignant primary brain tumors. Pediatric high-grade glioma (HGG) accounts for 8–12% of brain tumors and this is a devastating disease as 70–90% of patients die within 2 years of diagnosis. Recent sequencing of tumors revealed recurrent combinations of genomic and/or epigenetic aberrations associated with glioblastoma: meaningful subgroup classifications. Genetic lesions disrupting several epigenetic controllers at high frequency were found. Remarkably, the histone H3 variants H3.1 and H3.3 are frequently mutated in pediatric HGG, with up to 78% of diffuse intrinsic pontine gliomas (DIPGs) carrying K27M and 36% of non-brainstem gliomas carrying either K27M or G34R/V mutations. This comprises the first demonstration that histone mutations may be drivers of disease. High-frequency mutation of histone H3 to K36M were also found in chondroblastomas and to G34W/L in giant cell tumors of bone, which are diseases of adolescents and young adults. Intriguingly, histone H3K27M mutations are very rare in older patients however. What exactly do these mutations lead to? G34R/V mainly leads to the redistribution of H3K36me3, possibly by redirecting its enzyme SETD2—leading to enhanced expression of oncogenes, eg MYCN. H3K27M interferes with PRC2-EZH2 activity leading to global down regulation of H3K27me3 and reprogramming of epigenetic landscapes, including genome wide loss in H3K27me3, specific (aberrant) enrichment of the mark at several hundred genes and global DNA hypomethylation. H3.3-G34R/V mutation showed a 100% overlap with ATRX-DAXX mutations, unlike the H3.3-K27M mutation. Mutations in the DAXX (death domain-associated protein)/ATRX (alpha thalassemia/mental retardation syndrome X-linked protein) chaperone complex that loads H3.3 at pericentromeric and telomeric regions are associated with alternative lengthening of telomeres (ALT). This could be one mechanism that contributes to chromosome instability in cancer.

To gain insights into the exact mechanism by which these mutations trigger cancer, mouse and human models have been used. In particular, human embryonic stem cells to model pediatric gliomas with H3.3K27M histone mutation. Early neural progenitor cells (NPCs) were derived from human ESCs and co-transduce (lentiviral infection) a constitutively active form of PDGFRA, a shRNA against p53 and a HA-tagged H3.3K27 mutant. Expression of H3.3K27M led to a reduction in histone H3K27 trimethylation. Expression of H3.3K27M, or overexpression of PDGFRA, or p53 KD all show increased NPC proliferation.

Expression of mutant H3.3K27M leads to a developmental resetting of neural precursors to a more primitive stem cell state, which in combination with growth factor signaling, results in the acquisition or consolidation of oncogenic features.

In summary, a driver role for H3.3K27M mutation has been found in gliobastoma in the appropriate cell context and developmental window. Altered chromatin landscape induced by H3K27M facilitates the reacquisition of an earlier developmental program with subsequent activation of factors crucial to reprogramming and oncogenesis eg miRNA binding protein LIN28B. A chemical screen identified the menin pathway as a contributor to tumor maintenance. Menin is a tumor suppressor (mutated in patients with an inherited syndrome, multiple endocrine neoplasia type 1). MENIN interacts with histone H3 methyltransferases such as MLL to alter their activity.

Finally, the role of chromatin remodeling complexes in cancer was also discussed as many of the new putative driver mutations identified in tumor genomes were in these proteins. ATP-dependent chromatin remodeling is one of several mechanisms that permit the compaction and decompaction of DNA in the nucleus while retaining the capacity for replication, selective gene expression, and DNA repair and recombination. These complexes derive energy from the alternative ATPases Brg or Brm, which are paired with a second ATPase, β-actin. Nucleosomes thought to be the primary target of the complexes: in vitro transcription on nucleosomal templates: complexes can phase or position nucleosomes, exchange nucleosomes, induce nucleosome mobility, evict nucleosomes, or relax torsional stress possibly by direct actions on nucleosomes. At least 29 genes encoded by 15 gene families exist, with some subunits being highly tissue-specific. Importantly, genomic studies conducted on a number of human diseases have shown that the subunits most commonly mutated in human disease (cancer, neurodeveloppmental) are not those required for in vitro chromatin remodeling (nor for TF-directed targeting). Chromatin remodeling complexes have a far more important and instructive role in reprogramming and transformation than previously thought. BAF complexes may be guided to their targets by histone modifications and regional architecture, rather than just transcription factors bound to DNA. An “epigenetic-locus-recognizing” mechanism would provide a way of targeting complexes to loci which have specific features due to previous developmental events, thus enabling access to specific groups of genes with particular chromatin signatures.

More than 20% of human cancers bear a mutation to one subunit of 15-subunit of mSWI/SNF (BAF) complex. Mutations can be heterozygous or homozygous, somatic or germline, result in deletion point mutation, or translocation resulting in protein fusions. BAF Complexes can be oncogenes as well as Tumor Suppressors. Synovial sarcoma (nearly untreatable cancer of young people)—is always due to a t(X;18) translocation, fusion of part of SSX protein to SS18 BAF subunit. In the case of BAF47 (SMARCB1, INI1, hSNF5), this complex is always lost in malignant rhabdoid tumors (MTRs), which are primarily kidney tumor occurring in children (<2yrs). MRTs have the lowest mutation burden of all human tumors. In fact MTRs can be considered almost purely “epigenetic” tumors!

Other BAF subunits often show heterozygous mutation (dosage sensitive), mainly in adult cancers and very different tissues show mutations of specific subunits. BAF250A (ARID1A)—the most common BAF subunit mutation in cancer—is associated with ovarian clear cell carcinomas (dedicated to the complex; but NOT involved in in vitro chromatin remodeling). BAF57 (SMARCE1)—is only mutated in non-NF2 multiple spinal meningiomas. BRG ATPase—is mutated in >90% small cell ovarian cancers, but <5% small cell lung cancers. Various histological types are found though a significant number of tumors exhibit a peculiar clear cell morphology—which may be

linked to excessive glycogen accumulation as a consequence of abnormal carbohydrate metabolism. Many questions are open about BAF complex mutations in cancer. Why such tissue-specificity: Cell of origin? Why such dosage-sensitivity: Complex stoicheometry? How do mutated subunits affect cancer?

Mouse models are being generated to attempt to address some of these questions. A mouse conditional knock out model for human BAF47 mutation led to T cell lymphomas with very short latency. Although mouse model give different tumor type pathogenesis may be similar in fact. Indeed, BAF complexes in the mutant cells are unable to remove Polycomb complexes and H3K27Me3, from the Ink4a (Cdkn2a) locus, which normally suppresses proliferation. Nearly all of the effects of BAF47 loss could be explained by accumulation of polycomb and its products over the Ink4a locus indicated that polycomb inhibitors may be effective in these cancers. However mechanism by which loss of BAF47 leads to a failure to remove Polycomb is still unclear.

BAF also helps TopoII resolve tangled DNA, allowing it to segregate normally to daughter cells. Another possibility for the role of BAF complees is that when an oncogenic subunit of the BAF complex is mutated, DNA is not untangled at anaphase, leading to breaks with defective repair. As BAF subunit mutations prevent TopoII from contacting DNA, the prediction would be that cancers with BAF subunit mutations should be resistant to TopoII inhibitors. This may be helpful in guiding the use of these highly toxic inhibitors.

In conclusion, mutations in epigenetic regulators such as Polycomb associated H3K27 or BAF remodeling complexes, can have a widespread impact on gene expression and genome stability thereby producing multiple potential new phenotypes within a single tumor.

6. *Perspectives: Epigenetic biomarkers and therapies* (Perspectives : marqueurs et thérapies épigénétiques)

Discovery of new pathways / cellular processes in cancer

In this final lecture, I covered the potential therapeutic potential of epigenetic changes in cancer via targeted therapies, as well as their use as biomarkers. Cancers will eventually be classified based on their molecular (epigenomic and mutation) profiles in addition to their histologies. Integrated "–omics" information is even more powerful and information from genomes, transcriptomes and epigenomes. Indeed, important new insights from deep sequencing or single cell sequencing of different regions of tumors and over time.

Together with functional tests using model systems (mouse, iPS…), we can hopefully move towards a Systems Biology approach to cancer?

Before discussing therapies I covered some relevant recurring themes. First, epigenetic changes in tumors can often impair differentiation, they can block cells into a state of self renewal, and participate in their reprogramming. Abnormal epigenetic states can help lock in cell states that hinder the ability of cells to exit self renewal and differentiate normally Eg glioblastoma, colon cancer, leukemias—cells retain more primitive stem/ embryonic cell type. Epimutations may be induced by stress (replicative stress, inflammation etc). Epigenetic states can become aberrantly fixed—blocking tumor cells in self-renewing state. Epigenetic variation (due to metastable states) within a tumor can generate heterogenity and predispose to cancer progression. Most

new therapies focus on genetic abnormalities. Of the top 58 genes most often mutated in cancers, 16 encode epigenetic factors (writers, readers and erasers…). Cancer genome sequencing has allowed identification of specific driver mutations that can be targeted by simple molecules: this can provide robust initial responses but often has short durability with evolution of resistance. Many small molecule inhibitors to chromatin—associated proteins exist. DNA methylation inhibitors are amongst the oldest known epidrugs in clinical use. 5-AzaC first tested in the 1960's as a chemical to treat cancer but was highly toxic. Its potential for reversing epigenetic alterations was discovered in the 1970's in cultured cells—but clinical application only came later. Since the 1990s these drugs are used in hematologic malignancies, particularly for myelodysplasticsyndrome (MDS) (Decitabine). Efficiency in the clinic due to lowered dose—improving patient tolerance. Intriguingly, the most common set of genes induced by AZA in solid tumor cell lines are those involved in antigen presentation and interferon response. Patients who had previously received AZA for lung cancer subsequently had a highly efficient response to immune checkpoint inhibitors. Current thinking is that DNMT inhibitors probably sensitise cells by inducing an antiviral, anti-proliferative state, reactivating tumor antigen expression and altering cell signaling pathways. DNMT inhibitors induce a "viral mimicry" response in cells by activating endogenous retroviral repeats and upregulating immune signaling through secreted interferon, in addition to activating tumor antigen genes. 5-azacitidine and entinostat, which alter the epigenome, may prime patients' immune systems to respond to the checkpoint inhibitor. Pairing these drugs may radically improve patient outcomes and large clinical trials are currently ongoing.

Pharmacological inhibition of the histone H3K27me3 demethylase JMJD3 using GSKJ4 in DIPG orthotopic xenografts can reduce tumor growth and significantly extend animal survival. Analysis of treated tumors revealed decreased proliferation and increased apoptosis, relative to untreated control tumors. Thus results suggest that GSKJ4 anti-tumor activity is specific to K27M mutant tumors, both in vitro and in vivo, and its antitumor activity occurs in association with increasing K27me2 and K27me3 in tumor cells. In the case of acute myeloid leukemias (AML), which have a high frequency of mutations in R172 and R140 of IDH2, clinical trials ongoing with inhibitors of mutant IDH2 (eg reversible inhibitor AG-221) and seem promising.

Finally the greatest challenge in cancer therapy is to target drug resistant cancer cells. Low dose epigenetic drugs in combination with other therapies may be effective. The idea is that contrary to high-dose cytotoxic chemotherapy, where they proliferate unopposed, drug-resistant cancer cells may be at an evolutionary disadvantage in presence of low-dose chemotherapy owing to the high metabolic cost of their resistance mechanisms.

Séminaire en lien avec le cours – *Epigenetic targets in cancer*

Prof Kristian Helin (directeur du BRIC, Copenhagen, Denmark)

Colloque – *Epigenetics and cancer*

In depth presentations on the same topics as the Lectures, organized by Edith Heard & Hugues de Thé (BRIC, Copenhagen, Denmark)

Speakers/chairs include: Genevieve Almouzni, Steve Baylin, Stephan Beck, Manuel Estellar, Andy Feinberg, Jean Pierre Issa, Nada Jabado, Cigall Kadoch, Valérie Lallemand-Breitenbach, Raphael Margueron, Thomas Mercher, Paolo Salomoni, Eric Solary, Henk Stunnenberg, Anne Vincent-Salomon.

RECHERCHE

Depuis 2010, je dirige l'unité de Génétique et biologie du développement à l'Institut Curie composé de neuf équipes, dont la mienne. L'ambition de cette unité repose sur un concept simple, mais fondamental : mieux connaître les processus qui régissent le développement normal pour identifier l'origine des désordres pathologiques. L'unité de Génétique et biologie du développement fournit une trame multithématique et multidisciplinaire unique pour l'étude des événements qui affectent l'identité cellulaire dans un contexte développemental. J'anime une équipe de recherche au sein de l'unité de Génétique et biologie du développement à l'Institut Curie (Paris). Notre but est de comprendre comment, au cours du développement et de la différenciation cellulaire, l'acquisition de caractéristiques cellulaires spécialisées est assurée non pas par un changement de la nature et de la séquence des gènes, mais par la manière dont ces gènes sont exprimés. Le développement embryonnaire précoce des mammifères femelles s'accompagne de l'inactivation transcriptionnelle de l'un de leurs deux chromosomes X, achevant ainsi la compensation de dose vis-à-vis des mâles XY. Ce processus, connu sous le nom d'inactivation du chromosome X, représente un paradigme de l'épigenèse développementale. En étudiant le contrôle de l'inactivation du chromosome X, nous développons des méthodes et des techniques permettant la compréhension de mécanismes fondamentaux qui sous-tendent la régulation de l'expression des gènes, à la fois au cours du développement et de la différenciation cellulaire mais aussi lors de la tumorigenèse. L'inactivation du chromosome X est un modèle de choix pour décrypter les mécanismes moléculaires mis en jeu lors de la prise de décisions développementales, ainsi que pour assurer leur maintien. Notre recherche est organisée autour de quatre axes principaux de recherche :

• Quels sont les mécanismes contrôlant l'initiation de l'inactivation du chromosome X ?

• Comment la répression transcriptionnelle du chromosome X est-elle établie ?

• Comment l'état inactif est-il fidèlement transmis au cours des générations cellulaires ?

• Comment le développement tumoral affecte-t-il le maintien de l'état inactif du chromosome X ?

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