Cours I
“Une brève histoire du cancer : génétique et épigénétique”

“A Brief History of Cancer and Epigenetics”
Introduction: an epigenetic perspective of cancer

Why discuss cancer from an epigenetics perspective?

(1) The parallels with epigenesis and development are striking - understanding the differences is the challenge!

(2) Increasing connections between cancer and epigenetics in the last 50 years or so
Epigenetic therapies

(3) Recent new insights, thanks to genetics in model organisms, as well as genomics and single cell approaches, have led to exciting breakthroughs into our understanding of key roles that epigenetic modifiers play in cancer

Even Wikipedia today defines cancer as “a “process characterized by a progression of changes at the cellular, genetic, and epigenetic level that ultimately reprogram a cell to undergo uncontrolled cell division, thereby forming a malignant mass...”
Introduction: an epigenetic perspective of cancer

Cours 1 – Lundi 16h à 17h30, 29/02/2016:
"Une brève histoire du cancer : génétique et épigénétique »
“A brief History of Cancer and Epigenetics"

Cours 2 – Lundi 16h à 17h30, 07/03/2016 :
"La génomique et l'épigénomique des cancers : de la description aux mécanismes »
"Cancer Genomes and Epigenomics: from maps to mechanisms"

Cours 3 – Lundi 16h à 17h30, 14/03/2016 :
"Contrôle épigénétique des gènes et des génomes dans le cancer »
"Epigenetic control of genes and genomes in cancer"

Cours 4 – Lundi 16h à 17h30, 21/03/2016 :
"Voies épigénétiques du cancer - I »
"Epigenetic pathways in cancer - I”

Cours 5 – Lundi 16h à 17h30, 04/04/2016 :
"Voies épigénétiques du cancer - II »
"Epigenetic pathways in cancer - II"

Cours 6 – Mercredi 16h à 17h30, 06/04/2106 :
"Perspectives: Marqueurs et thérapies épigénétiques »
"Perspectives: Epigenetic Biomarkers and Therapies”
Cours suivi à 17h30-18h30 par un Séminaire
Prof Kristian Helin (Directeur du BRIC, Copenhagen, Denmark)

Le 9 et 10 Mai 2016
Colloque "Epigénétique et Cancer”
co-organisé avec le Prof. Hugues de Thé
Hippocrates (460-370 BC) : “Carcinos” - crab
Galen (130-200 AD) : “oncos” – swelling (gonflement)

Metastatic breast cancer in Siberian mummified woman
(>2500 BC)
‘Princess Ukok’ mummy in Anokhin museum, Gorno-Altaisk [Credit: Alexander Tyryshkin]

1500-3000 BC. Edwin Smith Papyrus, ancient Egyptian medical textbook

“Night”- 1526–1531, Michelangelo
San Lorenzo, Florence.
What is Cancer?

- Pathologists have tried to define cancer for more than a century
- Difficult to define cancer cells as they originate within tissues; and share many properties of normal cells
- We now know that cancer is not one disease but hundreds of different diseases
- One common characteristic of tumors, is uncontrolled increase in size - due to excess or autonomous cell division?

**All tumors consist of abnormal cells in which processes regulating normal cell division (and cell death) are disrupted**

- Cancer cells seem to become less dependent on signals from other cells; show altered tissue organization
- Malignant tumors can interact with their microenvironment, and can invade surrounding tissues
- Cancer cells can travel (metastasise) to other sites – and can sometimes (though rarely!) proliferate in new environments

**Tumors must be considered as organs: they are not just a collection of homogeneous cancer cells, whose entire biology can be understood by elucidating cell autonomous properties**

Cells from invasive micropapillary carcinomas (IMPCs) of the breast are characterized by a striking cell polarity inversion. Gruel et al, Breast Cancer Res. 2016 (Dr. A. Vincent –Salomon, Institut Curie)

Hanahan and Weinberg - 2011
Hypotheses to explain Cancer since the 19th Century

**Multiple Hypotheses**
- A problem of cell proliferation
- A problem of chromosome alterations
- An epigenetic process
- A problem of cell differentiation
- A problem of tissue organisation
- Tumor viruses
- Exogenous chemicals
- Altered chromosomes (and genes)
- Epigenetic alterations
Hypotheses to explain Cancer: uncontrolled cell growth

Virchow: Cancer is a problem of cell proliferation – unlimited cell division

- In 1845, first described and named (1847) leukemia
- Defined cancer using microscopy on specimens from autopsies
- In 1855, he proposed cancers arise by activation of dormant cells due to severe chronic irritation (inflammation?)
- Based on work of Remak, who had found convincing evidence that cells form by division, Virchow proposed (in Cellular Pathology published in 1858) that Omnis cellula e cellula ("All cells come from cells")
- Defined cancer as a disease involving uncontrolled cell growth

⇒ All cells, including cancer cells, are derived from other cells and cancer arises from uncontrolled cell growth
Hypotheses to explain Cancer: Abnormal chromosome constitution

Chromosomes were discovered by Walther Flemming in 1877

Leo Hansemann in 1890 proposed that irregularities of the mitotic process are responsible for disordered growth.....

Leo Hansemann’s drawings of abnormal mitoses in cancer tissue

Hypotheses to explain Cancer:
Abnormal chromosome constitution

In 1902, Boveri found that only sea urchin embryos possessing the full set of 36 chromosomes could develop normally. A "specific assortment of chromosomes is responsible for normal development and this can mean only that the individual chromosomes possess different qualities."

Boveri also realised that the Mendelian concepts of segregation and assortment could be interpreted to operate on a cellular level, with chromosomes containing Mendel’s so-called hereditary "factors”. In 1903 he wrote that “the characters dealt with in Mendelian experiments are truly connected to specific chromosomes.”

Drawing on von Hansemann's observations of abnormal mitotic figures in tumor cells and his own studies on sea urchin embryos undergoing abnormal mitotic divisions, Boveri proposed that an abnormal chromosome constitution may promote cancer.
Boveri’s theory (1902, and translated in 1915):

1. Cancer is a cellular problem.
2. Cancers originate from a single cell (...clonal evolution).
3. This primordial cell has, as a result of an abnormal process, a wrongly combined “chromosome complex.”
4. The chromosomal abnormality which is passed on to all the descendants of the cell of origin is the cause of rapid cell proliferation.
Boveri’s predictions for the causes of Cancer

Boveri’s visionary predictions from his 1902 monograph:

• Cell-cycle checkpoints (*Hemmungseinrichtung*: inhibitory mechanism) that would allow cell division only when a specific external stimulus is experienced by the cell.
• Tumour-suppressor genes (*Teilungshemmende Chromosomen*), the effects of which can be overcome by external signals, and which are physically lost in progressively growing tumours.
• Oncogenes (*Teilungsfoerdernde Chromosomen*) that become amplified (*im permanenten Übergewicht*) during tumour development.
• Tumour progression from benign to malignant, involving sequential changes of increased growth-stimulatory chromosomes and loss of growth-inhibitory chromosomes.
• The clonal origin of tumours.
• Genetic mosaicism.
• Cancer predisposition through inheritance of chromosomes (genes) that are less able to suppress malignancy.
• Cancer predisposition through inheritance of genes that cause aberrant mitoses.
• Inheritance of the same ‘weak chromosome’ from both parents leads to homozygosity for the defective chromosome and, consequently, to high penetrance cancer syndromes — for example, xeroderma pigmentosum.
• The role of wounding and inflammation in tumour promotion.
• Loss of cell adhesion in metastasis.
• Sensitivity of malignant cells to radiation therapy.

*From A. Balmain, Nat. Reviews Cancer (2001)*
Proof of Boveri’s theory: The Philadelphia translocation

*Nowell and Hungerford (1960)* discovered strong evidence to support Boveri’s hypothesis that a critical genetic alteration in a single cell, which provided the cell with a growth advantage, could give rise to a tumor (chronic myeloid leukemia CML – accounts for about 10% of all leukemias in US – *6600 new cases in 2015*).

First proof of a chromosome abnormality as a cause of cancer

Still not clear what causes it (exposure to high doses of radiation?)

Translocation leads to BCR-ABL fusion: the product of which is an abnormal kinase, stimulates proliferation of myeloid cells to produce CML

50 years later (2001) development of Gleevec, that can block the effects of this oncogene and stop progression of CML in 95% of patients.

Peter Nowell and David Hungerford, 1960
Epigenetic Hypotheses to explain Cancer

Since the time of Laennec, pathologists viewed cancer as acquiring properties of cells at different developmental stages, but appearing inappropriately in tumors.

Embryonic cell remnants remain in developing organs following embryogenesis cause cancer (Recamier, 1829, Remak, 1854, Cohnheim, 1875)


"Normal gene activity is misprogrammed by epigenetic mechanisms to produce a neoplastic pattern of metabolism in which all of the individual components are normal."

Cancer is a special expression of abnormal programming of gene function during cell differentiation
Epigenetics

Conrad Waddington (1942)

The study of the mechanisms of development through which genes bring about phenotypic effects.

Epigenesis (study of development) combined with genetics (the study of heredity)

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”. (Indeed - 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... => akin to a Darwinian process, tumor is an ecosystem and cells fight to survive... and proliferate.
Epigenetics

Conrad Waddington (1942)
The study of the mechanisms of development through which genes bring about phenotypic effects.

*Epigenesis* (study of development) combined with *genetics* (the study of heredity)

R. Holliday, A. Riggs and others (1970’s onwards)
Stable but reversible changes in gene activity, not due to DNA sequence differences.
(i) constitutes an observable difference between 2 cells with the same genotype (DNA)
(ii) this difference must persist in the absence of the initial signal
(iii) the difference must be heritable through mitotic division (or even meiosis).

Studies on processes such as X inactivation, imprinting, paramutation, position effect variegation made the link between epigenetics and DNA methylation, chromatin and non-coding RNAs *(See COURS 2013+2015)*
Disrupted chromatin organisation and aberrant epigenetic features in cancer cells – causes or consequences?

The Sex Chromatin in Human Malignant Tissues
K. L. Moore and M. L. Barr, 1957

Chaligné and Heard, Epigenetic defects in cancer (2014) Medicographia. 2014;36:293-299 (also see Chaligné et al, Genome Res. 2015)
Discovery of the Genetic Material and the Genetic Code

Oswald T. Avery, Colin MacLeod, and Maclyn McCarty (1944) demonstrated that DNA could function as the genetic material

The structure of the double helix
J. Watson and F. Crick (1953)

“It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.”

The Lac operon (1960): Gene control relies on specific repressors and activators and the DNA sequence elements they recognize.

E. Heard, 2016
Gene Regulation in Eukaryotes

Chromatin as a barrier and facilitator (COURS 2014, 2015)

Epigenetics: memorisation of gene activity states

Heritable (somatic) states (Epigenetic “Barriers”)

Altered Epigenome
Induced (eg environment, stress, damage or accidentally altered (eg aging)

Phenotypic consequences?

In the context of cancer:
- Genome instability
- Loss or change in identity?
- Uncontrolled cell growth?
- Invasion, Metastasis?

One Genome  Multiple Epigenomes
Gene expression & chromatin states

Different Cell Identities

DNA sequence-specific Transcription Factors & Signalling pathways
(positional information, cell-cell contacts, growth factors, etc
(to establish cell type, patterning, morphogenesis)
DNA Methylation: the first “epigenetic modification” to be explored

Work of Bestor, Bird, Jones and others in 1990’s discovered the enzymes that lay down and propagate DNA methylation; and the proteins that bind to methylated DNA (COURS 2013)

In 1975, Robin Holliday and Art Riggs independently postulated that:

1. DNA methylation might affect gene expression

2. Changes in DNA methylation could explain switching on & off of genes in development.

3. Predicted existence of enzyme(s) methylating a particular region of DNA – either by sequence specific binding, or via interaction with other sequence specific bound proteins

4. DNA methylation patterns could be heritable, if maintenance methylases existed that recognize hemi-methylated DNA soon after replication, but do not act on unmeth DNA ⇒ mechanism for heritability of the methylated and non-methylated DNA ⇒ heritability of a given pattern of gene activities
Inhibition of DNA Methylation could affect gene expression

Advent of the DNA methylation inhibitor 5-azacytidine (Jones, 1984), one of the first drugs to be used to treat cancer

Data on cultured mammalian cells showed that gene expression could be affected by methylation, and loss of DNA methylation could lead to gene reactivation and a change in cell identity

⇒ The inactive expression state of a gene could be stably maintained by DNA methylation (Razin and Riggs, 1980; Lock et al., 1987)

⇒ Robin Holliday went on to propose in 1987, that aberrant DNA methylation could sometimes lead to epimutations, or event mutations, for example in cancer...

⇒ NEXT LECTURE
Hypotheses to explain Cancer since the 19th Century

Multiple Hypotheses
- A problem of cell proliferation
- A problem of chromosome alterations
- An epigenetic process
- A problem of cell differentiation
- A problem of tissue organisation
- Exogenous chemicals
- Tumor viruses
- Altered chromosomes (and genes)
- Epigenetic alterations
Chemical induction of Cancer

Links between environmental agents and cancer had been made for centuries (tobacco and snuff, observations by Percivall Pott (1714-1788) chimney sweeps (ramoneur) and cancer of the scrotum, smoking...)

In 1950 Wynders and Graham conducted one of the first case-control studies suggesting a link between tobacco smoking and lung cancer. Similar conclusion were reached by Richard Doll and Bradford Hill: concluded that a chemical in tobacco smoke caused lung cancer, but were unable to explain the mechanism.

Other carcinogenic agents included X-rays, UV light, viruses, pollutants, and many other chemicals, including benzene, other organic solvents, and arsenic.

How they participate in cancer was not (in some cases still not) clear!
Viral Hypothesis to explain Cancer

In 1911, Rous had 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. ⇒ Cancer could be virally transmitted (Rous sarcoma virus, retrovirus),

F. Peyton Rous (1879-1972) 
(US pathologist & virologist)
Nobel Prize in Physiology or Medicine 1966 “For his discovery of tumour-inducing viruses”

New field of tumor virology; led to discovery of further tumor viruses
Laid the foundations of molecular mechanisms of carcinogenesis.

In his Nobel lecture, Rous stated “Despite protracted search, aided by the electron microscope, no tumor virus has ever been found in human milk, and family histories definitely rule it out.”

In humans, some tumor viruses exist - eg papilloma viruses discovered a couple of decades later.
The Golden Years of Molecular Biology…1960’s, 70’s, 80’s,

A convergence of work by geneticists, physicists, and structural chemists tackling a common problem: the nature of inheritance.

Approaches to clone DNA, to analyse it (restriction enzymes, southern blots) and eventually to sequence it, rapidly progressed.

Fred Sanger (1918-2013)
British biochemist
Two Nobel Prizes for Chemistry
For protein (1958) & DNA (1980) sequencing

E. Heard, 2016
From Tumor Viruses to the discovery of Reverse Transcriptase

In 1958, Howard Temin and Harry Rubin developed a quantitative “plaque” assay for studying Rous sarcoma virus in tissue culture, taking advantage of the rapid and reproducible way in which RSV transformed cells.

By the 1960s, the study of RNA tumor viruses had become a mature field encompassing both in vivo animal studies and tissue culture-based, bench-top experiments.

Temin went on to show that RNA tumor viruses replicated through a DNA intermediate, or provirus.

Temin and David Baltimore independently proved that virions of RNA tumor viruses contained RNA-dependent DNA polymerase activity: Reverse-Transcriptase (RT) => RNA could make DNA (“challenge” to central dogma!)

The discovery of RT was a watershed event in molecular biology, providing the means for generating cDNA and the key to reverse transcription–PCR (RT-PCR).

In recognition of their work, Temin and Baltimore received the Nobel Prize in 1975.
On December 23, 1971, President Richard Nixon signed the National Cancer Act and declared "war on cancer." The act was meant to strengthen the National Cancer Institute, which was established in 1937.

24 mars 2003 : Jacques Chirac lance le premier Plan de mobilisation nationale contre le cancer et en présente les 70 mesures....
Discovery of Oncogenes and Proto-oncogenes

In 1970, Duesberg and Vogt reported that the Rous Sarcoma Virus genome contained DNA sequences **not found** in the genomes of very closely related, **non-transforming** viruses.

In 1976, Stehelin, Bishop and Varmus demonstrated that this gene (Src) existed in the untransformed genomes of many organisms (including humans).

⇒ The **cellular** gene they discovered (c-src) was highly similar to RSV v-src
⇒ Thus the virus had obtained the gene sometime during replication in a different host to give rise to the v-src oncogene
⇒ Src is a **proto-oncogene**, which normally serves to activate cell division when the cell receives an appropriate signal; the mutant form (the **oncogene**) causes unrestrained activation of cell division...
Proto-oncogenes have a diversity of roles, controlling normal cell growth and division

<table>
<thead>
<tr>
<th>Nuclear</th>
<th>Cytoplasmic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Viral oncogenes</strong></td>
<td></td>
</tr>
<tr>
<td>SV40 large T</td>
<td>Polyoma middle T</td>
</tr>
<tr>
<td>Polyoma large T</td>
<td></td>
</tr>
<tr>
<td>Adenovirus Ela</td>
<td></td>
</tr>
<tr>
<td><strong>Cellular oncogenes</strong></td>
<td></td>
</tr>
<tr>
<td>myc, myb, N-myc</td>
<td>ras, src, erbB, neu,</td>
</tr>
<tr>
<td>p53, ski, fos</td>
<td>ros, fms, fes, fpsi, yes,</td>
</tr>
<tr>
<td></td>
<td>mtdraf, mos, abl</td>
</tr>
</tbody>
</table>

**The Action of Oncogenes in the Cytoplasm and Nucleus**

(Science 1985)

Robert A. Weinberg

Fig. 2. Three mechanisms by which oncogenes can allow a cell to escape dependence on exogenous growth factors: (A) by the autocrine mechanism; (B) by receptor alteration; and (C) by transducer alteration.
Proto-oncogenes have a diversity of roles, controlling normal cell growth and division

Oncogenes can act to:
- prevent cell cycle inhibition or overstimulate the cell cycle
- avoid cell death (apoptosis)
- prevent cell contact inhibition
- alter metabolism
- promote invasion

The era of oncogenes

Transfected DNA from a transformed cell could cause transformation in a normal cell (Shih et al, 1979)

Proto-oncogenes (point mutation in an endogenous proto-oncogene HRAS makes an oncogene (Tabin et al, 1982, Reddy et al; Taparowsky)

Chemical mutagenesis (mice) induces cancer through mutation of Ras proto-oncogene... (Balmain Pragnell 1983; Sukumar et al, 1983)

Altered proto-oncogene expression or function can turn them into an oncogenes....

However one oncogene was not enough in mammalian cells - combinations of multiple oncogenes required to transfrom normal cells into “tumor” cells...Land H, Parada LF, Weinberg RA. 1983. Nature 304:596–602

⇒ Could all tumors simply be caused by a few activating mutations – dominant oncogenes?

Predictions that Tumor Suppressors must also exist... ie that cancer could arise through the inactivation of recessive genes, that normally block cell growth

E. Heard, 2016
Predicting the existence of tumor suppressors

A General Theory of Carcinogenesis

DAVID E. COMINGS

Department of Medical Genetics, City of Hope National Medical Center,
Communicated by James V. Neel, July 26, 1973

ABSTRACT A general hypothesis of carcinogenesis is proposed consisting of the following features: (1) It is suggested that all cells possess multiple structural genes (Tr) capable of coding for transforming factors which can release the cell from its normal constraints on growth. (2) In adult cells they are suppressed by diploid pairs of regulatory genes and some of the transforming genes are tissue specific. (3) The Tr loci are temporarily activated at some stage of embryogenesis and possibly during some stage of the cell cycle in adult cells. (4) Spontaneous tumors, or tumors induced by chemicals or radiation, arise as the result of a double mutation of any set of regulatory genes releasing the suppression of the corresponding Tr genes and leading to transformation of the cell. (5) Autosomal dominant hereditary tumors, such as retinoblastoma, are the result of germ-line inheritance of one inactive regulatory gene. Subsequent somatic mutation of the other regulatory gene leads to tumor formation. (6) The Philadelphia chromosome produces inactivation of one regulatory gene by position effect. A somatic mutation of the other leads to chronic myelogenous leukemia. (7) Oncogenic viruses evolved by the extraction of host Tr genes with their conversion to viral transforming genes. As a result, in addition to the above mechanisms, tumors may also be produced by the reintroduction of these genes into susceptible host cells.

Heredity and Human Cancer

Alfred G. Knudson Jr, MD

Based on his epidemiological studies “Mutation and cancer: statistical study of retinoblastoma” Knudson, PNAS 1971.

Knudson’s two-hit hypothesis for tumorigenesis involving a tumour suppressor gene (TSG)

For historical perspective, see Kern et al 2002 “Whose hypothesis? Ciphering, sectorials, D lesions, freckles and the operation of Stigler’s Law.”
Discovery of tumor suppressors (1980s)

- Studies of familial, hereditary cancers (≈5% of all human cancers) revealed that the DNA defects transmitted through the germline were due to deletions in specific genes => loss of function

- Retinoblastoma gene (Rb) as 1st “tumor suppressor” gene (Friend et al, 1986) – subsequently found to be involved in regulation of cell division => Mutations affecting cell cycle regulatory genes became a major research focus

- p53 tumor suppressor: regulates both cell cycle progression and apoptosis. Hereditary p53 gene mutations can lead to Li-Fraumeni syndrome (LFS), increased risk of developing various types of cancers. Homozygous loss of p53 is extremely frequent in many cancers

Cells that are defective in p53 can still divide when they have DNA damage.

Cells lacking p53 fail to undergo apoptosis in response to agents that damage DNA

Loss of p53 interferes with apoptosis induced by other stimuli, such as growth factor deprivation and oxygen deprivation.
Discovery of tumor suppressors (1980s)

- Studies of familial, **hereditary** cancers (~5% of all human cancers) revealed that the DNA defects transmitted through the germline were due to deletions in specific genes => loss of function

- Retinoblastoma gene (Rb) as 1st “tumor suppressor” gene (Friend et al, 1986) – subsequently found to be involved in regulation of cell division => *Mutations affecting cell cycle regulatory genes became a major research focus*

- p53 tumor suppressor: Hereditary p53 gene mutations can lead to Li-Fraumeni syndrome (LFS), increase risk of developing various types of cancers. Homozygous loss of p53 is frequent in many cancers

- PTEN acts by opposing the action of PI3K, which is essential for anti-apoptotic, pro-tumorogenic Akt activation.

- BRCA proteins are multitasking: involved in DNA damage repair, regulation of gene expression (eg p53, and its target gene p21) => *Associated with genetic cancer syndromes (BRCA1 in 1994)*

Other examples of tumor suppressors include pVHL, APC, CD95, ST5, YPEL3, ST7, and ST14...

**Tumor suppressors were quickly shown to function in many key cellular processes (in tissue culture) including the regulation of transcription, DNA repair, cell-cell communication...**

Loss of function of these genes leads to abnormal cellular behaviour.

**However, in vivo precise roles of TS genes was less easy to define**

E. Heard, 2016
Can genetic alterations in proto-oncogenes & tumor suppressor genes explain cancer?

Most cancers were found to show 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. **Oncogenes drive cancer; tumor suppressors prevent it...**
Can genetic alterations in proto-oncogenes & tumor suppressor genes explain cancer?

Most cancers were found to show 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. **Oncogenes drive cancer; tumor suppressors prevent it...**

Colorectal cancer: *APC* 1st mutation “flowchart” of events (Fearon,Vogelstein, 1990, 1991) Mutations of the *APC* (adenomatous polyposis coli) gene are strongly associated with both inherited and sporadic cases of colon cancer. *APC*, like many tumor suppressors, functions to control the expression of genes critical in the cell division process

- A series of mutations in a cell causes 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.

**Is it really that simple?**
Somatic mutation model for the basis of cancer

The prevailing model for cancer development was that mutations in genes for tumor suppressors and oncogenes lead to cancer.

In most cases:

“Each tumor seemed a unique experiment of nature – acquiring a unique set of mutant genes in an unpredictable chronological order...” (R. Weinberg, Cell 2014)

Was this view too simple?
- Different cancers seem to involve very different sets of genes (except for specific hematological cancers)
- Somatic mutation rates could not explain the rapid evolution of many tumors
- Did not adequately explain the many chromosomal aberrations typical of cancer cells
- Failed to explain the genetic diversity among cells within a single tumor
- Does not explain frequent resistance to therapies

Alternative Models: Master genes controlling cell division? Chromosomal catastrophic events (“big leaps”)? Epigenetic models – epimutations and/or global epigenetic changes?

New insights from whole genome and epigenome sequencing... Next Week!
Epigenetic Models for Cancer

The Inheritance of Epigenetic Defects

ROBIN HOLLIDAY

Many rodent cell lines that lack particular enzymes or proteins are very stable and appear at first sight to have classical mutations in structural genes. However, 5-aza-CR treatments can induce massive reactivation of such genes, with as many as 10 to 30% of the survivors recovering enzyme activity, which represents about a millionfold increase over the spontaneous reversion rate. Mutagens or agents that damage chromosomes do not have these effects, and 5-aza-CR is itself only a weak mutagen in mammalian cells. Silent genes that have been reactivated by 5-aza-CR are listed in Table 1. These results provide strong evidence that genes are often inactivated in permanent cell lines by methylation, that the methylation is very stably inherited, and that 5-aza-CR results in demethylation of sites important for the control of gene activity. In several cases this has been confirmed by examination of the gene in question by means of methylation-sensitive restriction enzymes.

- Holliday proposed that heritable changes in gene activity could be due to DNA Methylation and that de novo methylation and 5-azaC treatment act to produce forward and reverse “Epimutations” in cell lines
- If DNA methylation is essential for the normal controls of gene activity during development, it follows that defects in methylation may have severe phenotypic consequences in diploid somatic cells.
- DNA me could act either by “shutting off” one or both alleles - or by inducing mutation
- Holliday favored the hypothesis that global DNA hypomethylation in cell lines could lead to mutation – such as chromosome rearrangements

Quite visionary as now we know that DNA me is key for controlling repeat elements – and unleashing of repeats may underlie chromosome instability -- at centromeres and also other repeats elements
Epigenetic Models for Cancer

Evidence for DNA me changes in cancer (pre-genome wide technologies)

Global DNA hypomethylation in cancer cell lines (Dilala and Hoffman, 1982; Ehrlich, 1982)

Local DNA hypomethylation at some oncogenes – eg Ras (Feinberg and Vogelstein, 1983)

DNA hypermethylation of CpG islands of tumor suppressor genes? (Jones and Baylin, 2002)
Epigenetic Models for Cancer

Evidence for DNA me changes in cancer (pre-genome wide technologies)

Global DNA hypomethylation in cancer cell lines (Dilala and Hoffman, 1982; Ehrlich, 1982)

Local DNA hypomethylation at some oncogenes – eg Ras (Feinberg and Vogelstein, 1983)

DNA hypermethylation of CpG islands of multiple tumor suppressor genes (Jones and Baylin, 2002)
Epigenetic Models for Cancer

• Both genetic and epigenetic views ultimately involve **abnormal gene expression**.

• The expression state of a gene is determined by presence of TFs and chromatin modifying enzymes, and the packaging of its DNA regulatory landscape.

• DNA mutations of tumor suppressors and/or oncogenes causes either loss or gain of function and abnormal expression.

• Epigenetic pathway to cancer is more complex – chromatin structure, DNA me, histone variants and modifications, nucleosome remodeling...Key aspect is potential **plasticity**

• The epigenome undergoes multiple alterations in cancer: genome-wide loss of DNA methylation, frequent increases in promoter methylation of CpG islands, changes in nucleosome occupancy, and histone modification profiles.

**How much of this is involved in early events – in “stem” or “progenitor” cells – or is just a consequence, and/or is involved in later events? **NEXT LECTURES!
Epigenetic Models for Cancer

Most genetic changes lead to all/nothing gene expression change
Epigenetic changes can lead to range of expression levels: help explain tumor heterogeneity?
Dosage may also be a key aspect for some proteins in selective advantage in cancer
Epimutations also useful as biomarkers and as therapeutic targets?
Understanding Cancer: from an Epigenetics perspective

- Mutation of epigenetic regulators
- Cis-sequence effects on epigenetic state

- 5mC deamination
- Chromatin effects on mutation & recombination
- Epigenetic silencing of DNA repair genes

- Long-range hypomethylation
- Focal hypermethylation
- Epigenetic activation
- Epigenetic silencing
- CIMP

Deregulating cellular energetics
Evading growth suppressors
Resisting cell death
Avoiding immune destruction
Genome instability & mutation
Epigenomic disruption
Inducing angiogenesis
Enabling replicative immortality
Activating invasion & metastasis
Tumor-promoting inflammation
Sustaining proliferative signaling

E. Heard, 2016
Epigenetics and Cancer

1. Are epigenetic changes simply a consequence of gene expression changes due to DNA sequence mutations and genomic instability in cancer?

2. Or might epigenetic changes **contribute** to cancer, by causing stable (potentially reversible) alterations in gene expression? (somatic – or even germ line?)

3. Can epigenetic changes induce mutations in cancer – cytosine deamination, or loss of repetitive element control, or aberrant silencing of DNA repair genes.

4. Can epigenetic changes contribute to tumor cell heterogeneity, and to the plasticity underlying phenotypic changes eg during invasion or metastasis.

5. How can a global knowledge of the epigenetic characteristics of cancer cells be used for translational purposes (diagnostic, prognostic, therapeutic...)?

E. Heard, 2016
Cours II

Lundi, 7 mars, 2016
16h à 17h30

La génomique et l'épigénomique des cancers :
de la description aux mécanismes

"Cancer Genomes and Epigenomics: from maps to mechanisms"