NEURO IMAGE

VOLUME 22, NUMERO 1
FÉVRIER 2006

NEURORADIOLOGY
MUHC–MNH

VOLUME 22, NUMBER 1
FEBRUARY 2006

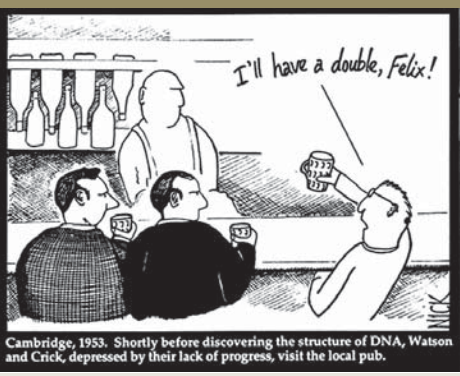
DNA

IN THIS ISSUE …

"DNA and brain tumours"

Kevin Petrecca & Marie-Christine Guiot

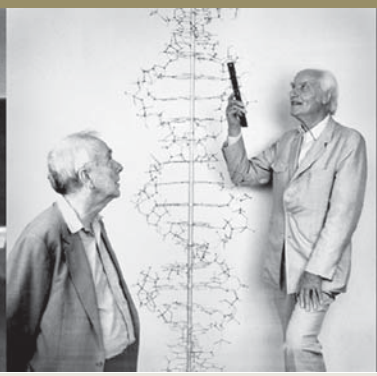
FROM A LASTING FRIENDSHIP CAME THE TRUE EVOLUTION OF THE DNA STRUCTURE



Cambridge, 1953. Shortly before discovering the structure of DNA, Watson and Crick, depressed by their lack of progress, visit the local pub.

INSPIRATION

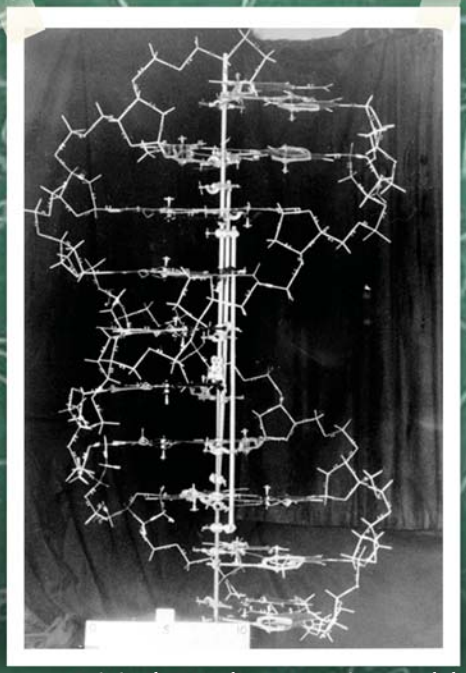
DEMONSTRATION



CELEBRATION APPLICATION

Maurice Wilkin's & Rosalind Franklin's X-ray image of DNA crystals which steer Watson and Crick toward their famous conclusion of the double helix seen below

http://www.mni.mcgill.ca/neuroimage/index.html



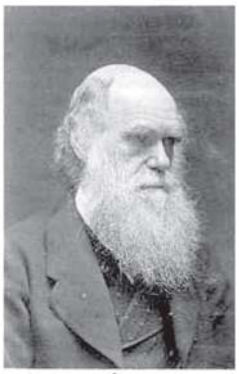
Original DNA demonstration model. Linus Pauling's blackboard is the background

Volume 22 – number1 – Bibliothèque nationale, ISSN 1180-0844 National Library of Canada, Production – Denis Melançon – Neurikon Inc.
Layout, Graphic Design by J.P. Acco at the Department of NeuroPhotography at the Montreal Neurological Hospital (11-2005)

Brain Tumors, Genetics, Epigenetics, and Treatment

Dr Kevin Petrecca

My handwriting same as Grandfather
– CHARLES DARWIN,
a scribble in the M notebook, 1838



Malignant gliomas are a collection of primary brain tumors that include astrocytomas, oligodendrogliomas, oligoastrocytomas and glioblastoma. They are the most common primary brain tumors and are among the most devastating of human malignancies. Standard treatment includes surgery, radiotherapy and chemotherapy. Complete surgical resection remains an effective means to provide a diagnosis and may prolong survival. However, surgical resection is not curative as the hallmark of malignant gliomas is macroscopically undetectable invasion of malignant glial cells into the surrounding normal brain, often significant distances from the primary tumor mass. Radiotherapy can prolong survival and is associated with significant morbidity. Recently, chemotherapeutic strategies have been shown to provide benefit to certain patient populations - depending on their genetic and epigenetic profile.

The human body consists of seventy-five to one hundred trillions cells. Almost all of these cells contain an entire genome - the complete set of inherited genetic information encoded in our DNA. The genome is composed of two types of DNA, coding (genes) and noncoding. It is now estimated that humans have about 30,000 genes, located in approximately 3 billion nucleotides. Genes, however, represent only three percent of human DNA; the rest is noncoding DNA. Within these noncoding regions of the genome is the information that determines when and where genes are active.

THE BIRTH OF GENETICS, PRE – 1945

Circa 3000 BC
Ancient Chinese and Sumerian farming techniques selectively breed crops and animals.

1866
Gregory Mendel publishes his work on the heredity of peas, in which he notes certain factors, later called genes, are passed from parent to offspring.

1869
Working with soiled bandages, Friedrich Miescher identifies DNA as an acidic substance in cell nuclei. It is at first called "nuclein."

1900
Hugo deVries, Carl Correns, and Eric von Tschermak independently confirm Mendel's work.

1911
Thomas Hunt Morgan shows that genes are located linearly along chromosomes.

1928
In an experiment with lab mice, Frederick Griffith transfers the deadly component of a strain of pneumonia bacteria to an innocuous bacteria strain, and determines there must be a genetic "transforming factor" in the bacteria.

1929
Phoebus Levene discovers that the sugar deoxyribose is present in nucleic acids and later shows that DNA is made up of nucleotides, which are made up of a deoxyribose sugar, a phosphate group, and one of four bases.

1943
William Astbury takes first X-ray diffraction images of DNA.

1944
Oswald Avery, Colin MacLeod, and Maclyn McCarty show that DNA, and not protein, is the "transforming factor" Griffith first identified.

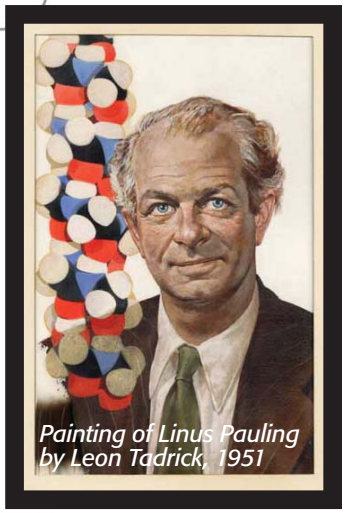
Cancer, now the leading cause of death in North America, is a genetic disease. Although coding DNA provides the majority of the known culprits, noncoding DNA has emerged a very relevant player. Genes responsible for the development of cancer fall into three broad categories: oncogenes, tumor-suppressor genes and stability genes. To date, no single genetic abnormality has been shown to cause cancer, rather only when several genes are defective does a malignant cancer develop. Thus it is best to think of mutated cancer genes as contributing to, rather than causing, cancer.

ONCOGENES

Oncogenes are genes that when altered can produce a gene product that is constitutively active – similar to a stuck accelerator in an automobile; the car still moves forward even when the driver removes his foot from it (Vogelstein et al., 2004). Oncogene alterations can result from chromosomal translocations, gene amplifications or subtle mutations affecting crucial residues that regulate the activity of the gene product. An activating somatic mutation in one allele of an oncogene is generally sufficient to confer a selective growth advantage on the cell. Thus oncogenes can be thought of as genes that promote cell growth under normal circumstances, but, when altered, promote growth in an unregulated, pathological manner.

TUMOR SUPPRESSOR GENES

Mutations in tumor-suppressor genes lead to a reduction in the activity of the gene product. Such inactivations arise from missense mutations that alter the function of a protein or from epigenetic silencing. A mutation in a tumor-suppressor gene is similar to a dysfunctional brake in an automobile; the car doesn't stop even when the driver attempts to engage it (Vogelstein et al., 2004). Mutations in both the maternal and paternal alleles of a tumor-suppressor gene are generally required to confer a selective advantage to the cell. This situation commonly arises through the deletion of one allele via a gross chromosomal event—such as loss of an entire chromosome or chromosome arm, eg., loss of heterozygosity (LOH) of 1P and 19q in oligodendrogliomas, coupled with an intragenic mutation of the other allele. Thus tumor suppressor genes can be thought of as genes that reduce cell growth under normal circumstances, but, when altered, result in a loss of growth inhibition.



Painting of Linus Pauling by Leon Tadrick, 1951

STABILITY GENES

A third class of cancer genes, called stability genes or caretakers, promote tumorigenesis in a completely different way when mutated. These genes are responsible for repairing mistakes made during normal DNA replication or induced by exposure to mutagens. Stability genes keep genetic alterations to a minimum, and thus when they are inactivated, mutations in other genes occurs at a higher rate. All genes are potentially affected by the resultant increased rate of mutation, but only mutations in oncogenes and tumor-suppressor genes affect net cell growth and can thereby confer a selective growth advantage to the mutant cell. As with tumor-suppressor genes, both alleles of stability genes generally must be inactivated for a pathological result. In the analogy to autos, stability genes represent the mechanics and a defective stability gene is like an incompetent mechanic that is unable to fix an engine problem (Vogelstein et al., 2004).

FINDING DNA, 1945 – 1965

1948-1949
Linus Pauling suggests sickle cell anemia is caused by a defect in the molecular structure of protein, and later describes the shape of certain proteins as an alpha helix.

1950
Erwin Chargaff shows there are equal amounts of the nucleotides adenine (A) and thymine (T) as well as equal amounts of guanine (G) and cytosine (C), i.e. there is an A for every T and a G for every C.

1952
Alfred Hershey and Martha Chase show that viral DNA and not viral protein direct the replication of new viruses, confirming that DNA is the molecule that mediates heredity.

Maurice Wilkins and Rosalind Franklin take X-ray images of DNA crystals which will steer Watson and Crick toward their famous conclusions.

1953
James Watson and Frances Crick describe the three-dimensional structure of DNA as a double helix: two spiraling strands held together by complimentary base pairs.

1959
Jerome Lejeune determines that Down's syndrome results from an extra chromosome -- a total of three copies of chromosome 21.

TUMORIGENESIS

Mutations in these three classes of genes can occur in the germline, resulting in hereditary predispositions to cancer, or in single somatic cells, resulting in sporadic tumors. The first somatic mutation in an oncogene or tumor-suppressor gene initiates the neoplastic process by generating a clonal cellular expansion – a collection of cells generated from a single progenitor cell that are genetically identical. Subsequent somatic mutations result in additional rounds of clonal expansion, and thus tumor progression. Indeed, the best modern definition of a neoplastic cell is one that has clonally expanded as a result of somatic mutations. Germline mutations of these genes cause cancer predisposition, not cancer per se: people with these mutations have a 'head start' on the neoplastic process, as a mutation that can contribute to cancer is already present in every one of their cells. Such individuals therefore often develop multiple tumors that occur at an earlier age than in individuals whose cancer-gene mutations have all occurred somatically. In people with these syndromes, only a very small fraction of the total cells in an at-risk organ become neoplastic because other (somatic) mutations are required to develop a clinically significant lesion.

HOW DOES GENETIC INFORMATION GUIDE TREATMENT?

Genotyping, the identification of genetic abnormalities, has been influencing treatment the treatment of brain tumors for the last decade. This is especially true for oligodendroglioma (Louis et al., 2002). Approximately 60% of anaplastic oligodendrogliomas respond to a chemotherapy regimen of PCV (procarbazine, CCNU, and vincristine) – an alkylating agent. Genotyping can be used to predict which group of patients will fall into this

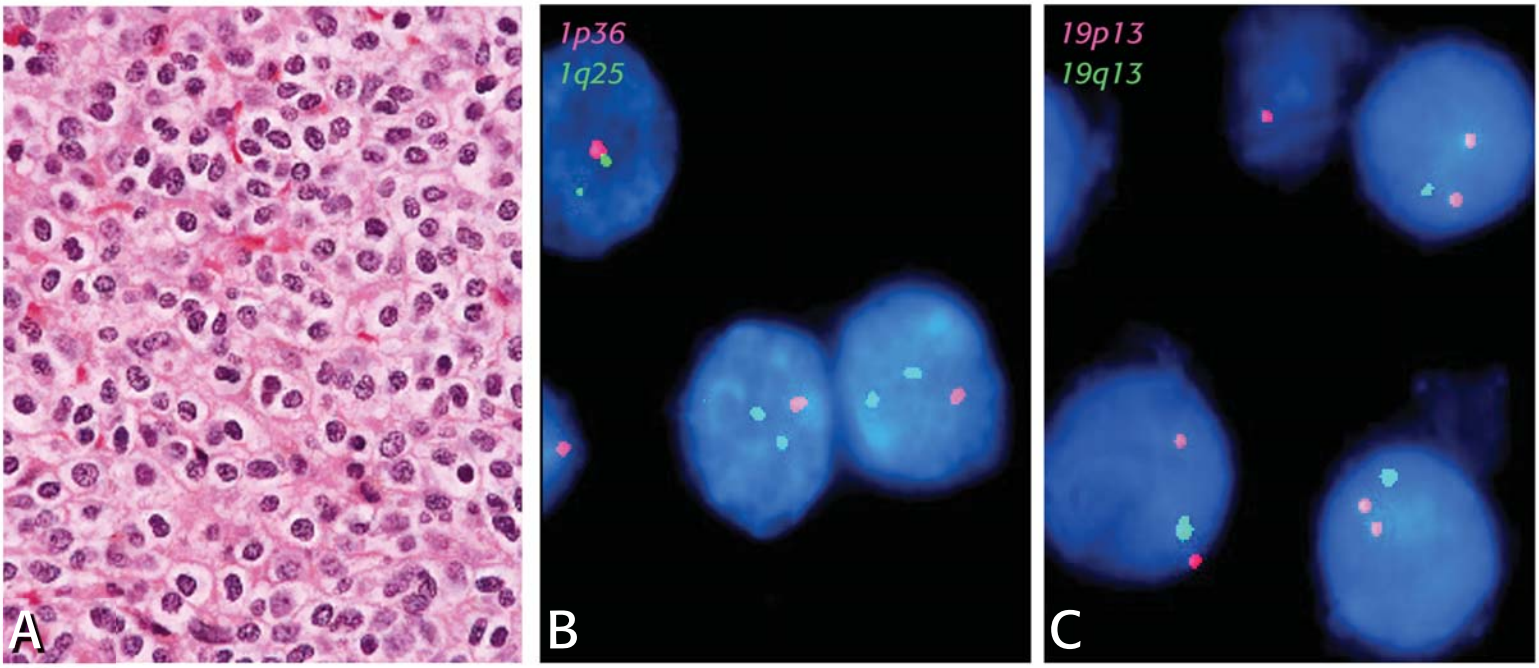


Figure 1: Oligodendroglioma grade II (WHO) with LOH 1p and 19q. *(A) H&E stain showing the typical "fried egg" appearance. (H&E stain). (B) Fluorescence In Situ Hybridization (FISH) for Chromosome 1p: The cells show 1 red signal and 2 green signals indicating a deletion of 1p36. (C) FISH for chrosome 19q: The cells show 1 green signal and 2 red signals indicating a deletion of 19q13.*

GENETIC ENGINEERING: 1965 – 1985

1966
Marshall Nirenberg, H. Gobind Khorana, Francis Crick, George Gamow, and other scientists crack the genetic code -- sixty-four nucleotide triplets that constitute a universal genetic code for all cells and viruses.

1968
Werner Arber isolates restriction enzymes.

1972
Drawing on the work of Stanford graduate student Peter Lobban and his faculty advisor Dale Kaiser, Paul Berg employs restriction enzymes to cut and splice DNA, creating the first strand of recombinant DNA.

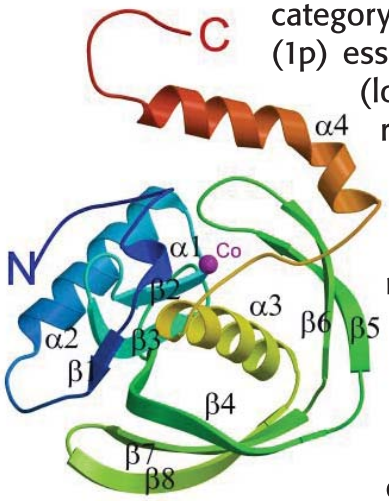
1977
Fred Sanger and others sequence DNA.

1980
U.S. Supreme Court decision allows genetically modified organisms to be patented.

1982
First genetically engineered drug: Humulin, a kind of insulin grown in genetically modified bacteria is produced by the Eli Lilly pharmaceutical company.

1983
James Gusella uses blood samples collected in Venezuela by Nancy Wexler to identify the Huntington's disease marker.

1985
PCR, to date the most accurate and sensitive method for amplifying DNA, is developed by Kary Mullis.



category. Those patients whose tumors have lost the short arm of the first chromosome (1p) essentially always respond to PCV, and those with combined loss of 1p and 19q (long arm of chromosome 19) and lack other detectable alterations have durable responses and long survival times - over 10 years. In contrast, those whose tumors lack these genetic alterations but harbor others such as EGFR amplification rarely respond to PCV in a durable manner and have short survivals - less than 2 years. Hence, genotyping can direct the former group (1p loss) to initial PCV instead of radiotherapy, the latter group (1p intact) can bypass the difficulty of PCV therapy, in favor of radiotherapy, which causes few side effects in patients with short survival times. It is not known which specific gene(s) loss on 1p and 19q renders susceptibility to alkylating agents giving rise to this therapeutic advantage.

Are there other opportunities to target chemotherapies to specific patient populations? Yes – the mechanism of resistance to alkylating and methylating agents has been determined (Esteller et al., 2000). Alkylating (and methylating) agents cause cell death by binding to DNA, usually the O6 position of guanine. The addition of this alkyl or methyl group to the DNA backbone results in the formation of lethal cross-links between adjacent strands of DNA. The cross-linking of double-stranded DNA is inhibited by the cellular DNA-repair protein O6-methylguanine-DNA methyltransferase (MGMT). The MGMT protein reverses alkylation at the O6 position of guanine, thereby averting the formation of cross-links.

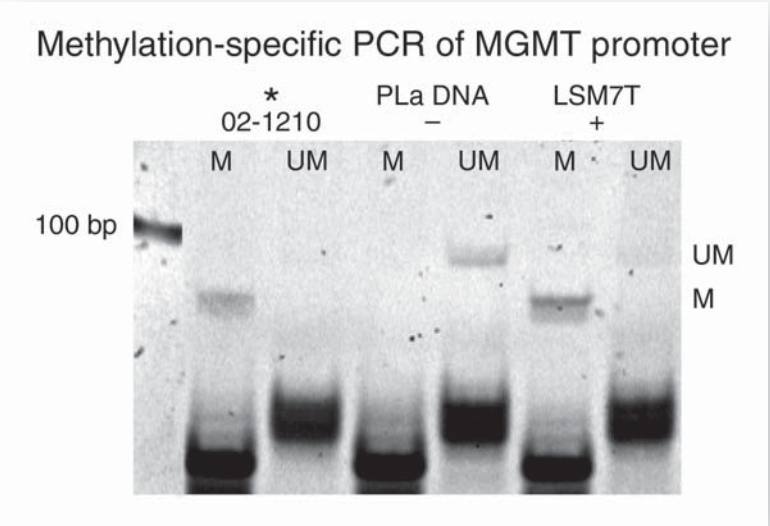
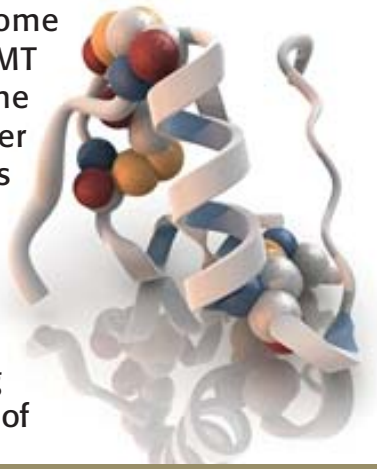


Figure 2: Methylation Specific PCR (MSP) for MGMT gene promoter. *Bisulfite- modified DNA was amplified by PCR using primers that were specific for methylated or unmethylated sequence of the gene. The tumor (02-1210), Anaplastic Astrocytoma shows a methylation of the promoter gene of MGMT.*

MGMT expression is not uniform in all brain tumors. Approximately 30 percent of gliomas lack MGMT. This deficiency may increase the sensitivity of brain tumors to alkylating agents. The MGMT gene (chromosome 10q) is not commonly mutated or deleted in brain tumors. Absence of MGMT expression is caused by epigenetic phenomena - changes that do not alter the genetic information of the cell. In this case, methylation of the MGMT promoter leads to gene silencing – an absence of MGMT gene expression and thus protein expression.



How is MGMT expression status useful? A recent phase 3 trial demonstrated that among patients with glioblastoma whose tumor contained a methylated MGMT promoter, a survival benefit was observed in those treated with temozolomide and radiation; median survival was 21.7 months as compared to 15.3 months among those who were assigned to radiation alone (Hegi et al., 2005). In the absence of

SEQUENCING THE HUMAN GENOME: 1986 – 2003

1986
First discussions of initiating a Human Genome Project.

1989
Francis Collins and Lap-Chee Tsui identify the Cystic Fibrosis gene.

1990
First successful gene therapy, performed on a girl with an inherited immune deficiency disorder.

1993
Huntington's disease gene is identified.

1994
The first genetically engineered food is approved by the FDA.

1996
Dolly the Sheep is the first mammal to be cloned.

2000
June 26: Joint announcement of a completed "working draft" DNA sequence of the human genome by the public project and the private company, Celera.

2003
April 15: Announcement of the final completion of the human genome sequence.

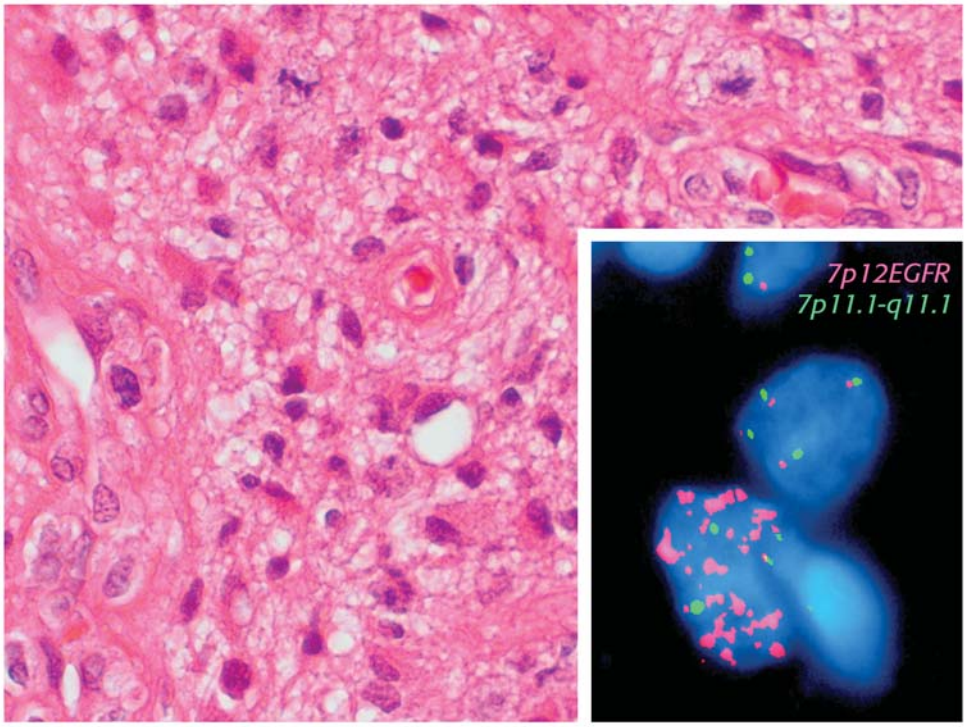


Figure 3 Amplification of EGFR gene in a Glioblastoma Multiforme. *H&E showing an area of a GBM with nuclear pleomorphism and mitotic figures. The cells show numerous red signals corresponding to 7p12 EGFR and only 2 green signals (7p11.1-q11.1) indicating an amplification of EGFR gene.*

methylation of the MGMT promoter, there was a smaller and statistically insignificant difference in survival between the treatment groups (12.7 versus 11.8 months). Thus determination of MGMT promoter methylation status may allow the selection of patients most likely to benefit from alkylating/methylating chemotherapeutic strategies.

CONCLUSION

Uncovering the genetic and molecular basis of cancer has strengthened our understanding of cancer formation and progression. Similarly, our approach to the treatment of this disease has changed from a common therapeutic approach for a certain type of cancer to one that is tailored to the genetic profile of each tumor. The integration of the genetic profile of the tumors has been a priority in the Department of Neuropathology at the MNI/MNH. It started 6 years ago with LOH 1p and 19q in Oligodendrogliomas, and progressively as new clinical studies are published and new markers become relevant to therapy, we developed these new molecular tests in our laboratory. At this date, LOH 1p and 19q, amplification of EGFR gene and methylation status of MGMT gene are the markers the most often requested by the Oncology team. Further advances in our understanding will undoubtedly improve treatment options and patient selection.

References

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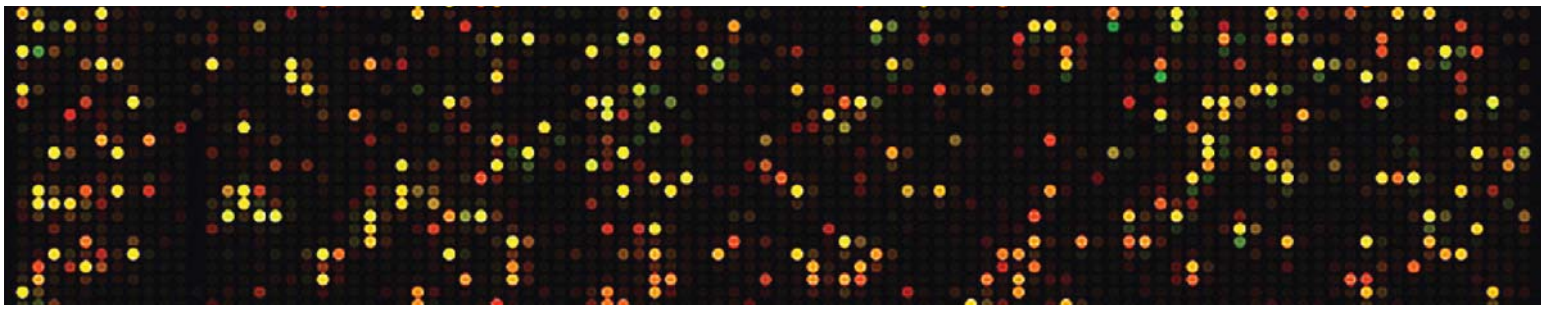
(3) Esteller M, et al. Inactivation of the DNA-Repair Gene MGMT and the Clinical Response of Gliomas to Alkylating Agents. *The New England Journal of Medicine*. 343;19,2000.

(4) Hegi ME, et al. MGMT Gene Silencing and Benefit from Temozolomide in Glioblastoma. *The New England Journal of Medicine*. 352;10,2005.

(5) Timeline is from http://www.pbs.org/wnet/dna/timeline

(6) **Figures for LOH1p/19q, methylation status and EGFR amplification provided by Marie-Christine Guiot, Neuropathology**



2003 DNA £2 Commemorative Coin from the Britsih Royal Mint, commemorating the 50th anniversary of James Watson and Francis Crick's achievement in identifying the structure of DNA, beautifully captured in John Mills' design.

DNA & LIFE

DNA is the basis for creation of any living being. It carries the mystery of how we, human beings, have been made.

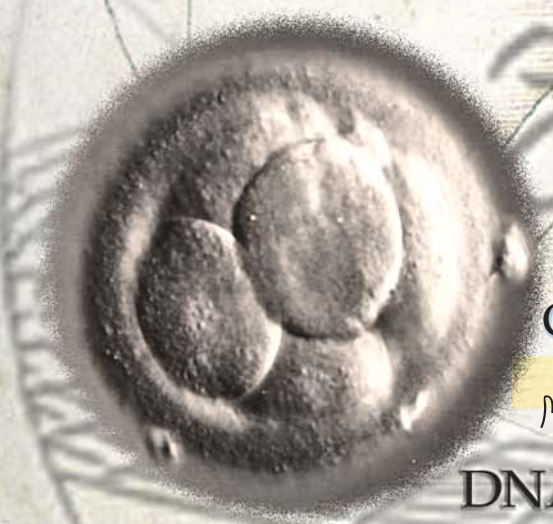
此致

Amicalement
Best Regards
Herzliche Gruesse
O Genki De
Saluti affettuosi
Saudações
Filika

The DNA in every cell contains the total body information.
The specifity of DNA is 10^{78000}
In a universe only 10^{18} seconds old it is obvious
that life could not have evolved by chance
– David Foster,
The Philosophical Scientists, 1985

Namaste
As-salaam alaykum
Bäst Hälsningar
Cordialmente
Afectuosamente
Respetos
敬意

The most beautiful thing we can
experience is the mysterious.
It is the source of all art and science.
– Albert Einstein



Evolution versus
Intelligent design:
Continuing the dialogue
info@metanexus.net

DNA and LIFE.... DNA and LIFE AFTER LIFE....