Mammalian Genomic Imprinting and Cancer

“Mom’s and Dad’s genes are NOT created equal”

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Oncology for Scientists I
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Objectives

- Define Epigenetics & Genomic Imprinting
- Discovery
- What is the “imprint”
  - DMRs and ICRs
- Lifecycle of an Imprint
- 2 main mechanisms of imprinting
  - Insulator and long non-coding RNA
- Genomic Imprinting and Cancer
The study of mitotically or meiotically heritable changes in gene expression or cellular phenotype, caused by mechanisms other than changes in the underlying DNA sequence.
DNA methylation:
- Epigenetic modification that adds a methyl group
- Occurs almost exclusively at CpG dinucleotides
- Generally results in gene repression
- Tightly linked with Histone modifications

CpG islands
- Mainly found at promoter regions
- Generally unmethylated – except at imprinted genes
- Methylated CpG islands associated with closed chromatin

DNMTs: add methyl groups to DNA
- Dnmt1 – maintenance methyltransferase
- Dnmt3a/b – de novo methyltransferase

Chromatin (Histone) Modifications
- Euchromatin: low DNAme, acetylated histones, permissive to gene expression
- Heterochromatin: high DNAme, deacetylated and methylated histones, repressive
Genomic Imprinting

Definition...

- “An epigenetic process leading to parental–specific expression of one to two percent of mammalian genes” – Barlow, 2011
- “An epigenetic process that can involve DNA methylation and histone modulation in order to achieve monoallelic gene expression without altering the genetic sequence” – Wikipedia
- “One of the 2 parental alleles is epigenetically silenced” – Brideau et al 2010
Important Terms:

- **Biallelic expression**: a gene is expressed from both the maternal and paternal alleles. You have 2 copies of the gene
  - Most genes

- **Monoallelic expression**: A gene is expressed from only one of the parental alleles. You have only one copy of the gene
  - Imprinted genes

- **Differentially Methylated Region (DMR)**: Methylated on one parental allele and not the other.
Imprinting Defies Typical Mendelian Genetics

- Biallelic Expression
- Maternal Expression
- Paternal Inactivation
  - Paternal Expression
  - Maternal Inactivation

- Maternal:
  - 2 copies
- Paternal:
  - 1 copy
  - 1 copy
Imprinted Genes

- Approx. 100 loci imprinted in human and mouse
- Associated with Differentially methylated regions (DMRs)
- Involved in growth regulation and development
  - MAT expressed genes: Growth suppressors (TSGs?)
  - PAT expressed genes: Growth promoters (Oncogenes?)
- Occur in Clusters that share common Imprint Control region (ICR) (more on this later)
- Imprint status varies between tissues, developmental stages and species
“Mom’s and Dad’s genes are NOT created equal”

Discovery of Genomically Imprinted Genes
“Mom’s and Dad’s genes are **NOT created equal**” : Pedigree of imprinted maternally expressed phenotype
Disruption of identical genes on chr.15 leads to different disorders depending on which parental chromosome the disruption is inherited from.

- **PWS**: genes lost on the PAT chromosome
- **AS**: genes lost on MAT chromosome

“Mom’s and Dad’s genes are NOT created equal”
“Mom’s and Dad’s genes are NOT created equal”

What do you get when you cross a horse and donkey?

- **Hinny**: Donkey $\times$ Horse
- **Mule**: Horse $\times$ Donkey
Discovery... Nuclear Transfer Experiments
(1980’s Solter and Surani)

Fertilized diploid embryo (zygote)

Removed pronucleus and generated zygotes with two MAT or two PAT nuclei

Gynogenetic lethal
Wildtype viable
Androgenetic lethal

Barlow and Bartolomei, 2007
The two parental genomes provide reciprocal functions.
Evidence of Imprinted Genes: Uniparental Disomy (UPD)

- When the cells of an offspring receive both copies of a particular chromosome or chromosome segment from the same parent
  - Paternal UPD: both copies from father
  - Maternal UPD: both copies from mother
  - Segmental UPD: Only part of a chromosome inherited from same parent
Evidence of Imprinted Genes: Mouse embryos with UPD for certain chromosomes or regions showed developmental failure.

Demonstrated the existence of at least 9 chromosome regions on 6 mouse chromosomes that showed an imprinting effect (i.e., contributions from both parents necessary).
What is the mark or “Imprint”

“...the non-genetic difference that distinguishes the two parental genomes...”

1. Must be able to influence transcription
   - Repress or activate gene expression on one allele

2. Must be heritable in somatic lineages
   - Pass on to daughter cells

3. Must be a way to erase the mark
   - Your germ cells need to have the correct mark to pass on to offspring:
     - Paternally inherited chromosomes in female germline need to establish paternal specific mark and visa versa.
DNA Methylation as the “imprint”

1. Must be able to influence transcription
   ◦ 5–MeC at CpGs can influence transcription

2. Must be heritable in somatic lineages
   ◦ DNMT1 – maintenance methyltransferase – recognizes hemimethylated DNA (present at replication forks)

3. Must be a way to erase the mark
   ◦ Germ–cell specific genome–wide reprogramming in the developing embryo (active & passive demethylation)
Differentially Methylated regions (DMR)

Control expression of one or more imprinted genes in a cluster

Often associated with long non-coding RNA promoters

Definative proof that something is an ICR: KO → Loss of Imprinting (LOI) of one or more distant genes
Knockout of ICR leads to LOI

This ICR is already repressed due to DNAme so must be deleted on MAT allele to see effect.
Lifecycle of an Imprint
The “Life Cycle” of an Imprint—following a maternally expressed imprinted gene

Once erased how does the methylation machinery know where to act to establish sex-specific DNA methylation?

Falls et al. 1999, Am J. Path. 154: 635
What makes imprinted DMRs targets for the de novo methylation machinery?

- **Sequence features**
- **Specific chromatin signature at DMRs**
  - may mediate DNA methyltransferase access to DNA.
- **Transcription through the DMR**
  - Most known maternally-methylated DMRs are located within transcription units
  - May be required to create or maintain open chromatin so the DNAme machinery has access
How do ICRs regulate imprinted genes?

- Insulator (enhancer blocking) model
- Non-coding RNA model
Enhancer-blocking activity of chromatin insulators

Bell, A. & Felsenfeld, G. Current Opinion in Genetics (1999) 191-198
CTCF: CCCTC Binding Factor

- Highly conserved 11ZF nuclear phosphoprotein
- DNA targets recognized by combinatorial usage of 11 Zn fingers
- Protein interaction domains
- Transcriptional activation, repression, silencing and chromatin insulation

Ohlsson, Renkawitz, Lobanenkov (2001) TIGs
**Insulator Model: Imprinting of the Igf2/H19 genes**

- **Female (♀)**:
  - **IGF2** is on the paternal allele.
  - **H19** is on the maternal allele.
  - **H19 DMR insulator** allows expression of both **IGF2** and **H19**.

- **Male (♂)**:
  - **IGF2** is on the maternal allele.
  - **H19** is on the paternal allele.
  - **H19 DMR insulator** is absent, leading to the expression of only **H19**.

**H19 DMR is able to regulate the imprinting of both H19 and IGF2**
More realistic model?

CTCF binds to unmethylated maternal allele of H19/Igf2 ICR

modified from Phillips and Corces, 2009
Lnc RNA Model

Xist in X chromosome inactivation
Airn at the Igf2r locus
Kcnq1ot1 at the KvDMR1 locus
Long-non-coding RNA (lncRNA):
- Non-protein coding transcripts longer than 200 nucleotides

Ferguson-Smith, 2011 Nature
How do lncRNAs silence genes in cis?

- ncRNA may “recruit” repressive chromatin modifier complexes that suppress transcription (e.g. polycomb group [PcG] proteins).

- ncRNA may “target” the chromosome domain to a nuclear compartment that is devoid of RNA polIII and enriched in PcG proteins or may help set up the compartment and bring along the imprinted domain.

- the act of transcription (of the ncRNA) through the domain may affect the binding of a regulatory protein complex.
Genomic Imprinting and Cancer
Hydatidiform moles arise from androgenetic (46 chromosomes all of paternal origin) embryos.

Ovarian teratomas: germ cell tumors that arise from parthenogenetic (46 chromosomes all of maternal origin).

These tumors indicate that an imbalance between maternal and paternal genomes causes neoplasia.
Mice engineered to be “imprint–free” are tumor prone

Normal imprinting of some genes is disrupted in tumors

Beckwith–Wiedemann Syndrome (BWS): a cancer predisposition syndrome results from abnormal expression of imprinted genes
made ES cells that could be manipulated to:
  ◦ inactivate \textit{Dnmt1} and thus lose all methylation and then turn \textit{Dnmt1} back on
  ◦ found that global methylation was restored but methylation at imprinted genes and ICRs was not
  ◦ described as imprint–free (IF)

used these IF ES cells to make chimeric mouse embryos from which they derived IF mouse embryo fibroblasts (MEFs)
  ◦ these IF MEFS were found to become \textit{spontaneously immortalized at high frequency}; they were easily transformed (using cooperating viral oncogenes)

100% of chimeric adult mice made with imprint–free ES cells developed tumors; tumors were derived from IF–ES cells

concluded that Loss of Imprinting (LOI) alone can predispose cells to tumorigenesis by leading to immortalization which lowers the threshold for transformation

Holm et al. Cancer Cell 8:433 (2005.)
Loss of Imprinting (LOI) in Tumors
Loss of imprinting of Tumor Suppressor and Growth Promoting genes

TSG  Tumor Suppressor Gene
GPG  Growth Promoting Gene

Remember:
Maternally expressed: Growth suppressors
Paternally expressed: Growth promoters
IGF2

Potent fetal mitogen (growth stimulator) and survival (apoptosis antagonist) factor. LOI leads to double dose of Igf2

How is Igf2 imprinting deregulated?
LOI of H19/Igf2 via deletion of ICR leads to double dose of Igf2, a potent growth factor.
LOI of the Igf2/H19 genes by abnormal methylation of maternal H19 ICR

DNMT

hypermethylation during carcinogenesis results in
LOI of the Igf2/H19 genes by abnormal methylation of maternal H19 ICR

Now we have 2 copies of this growth promoter (oncogene)!!!
BORIS (CTCFL, CTCF–like) (Brother Of Regulator of Imprinted Sites)

- Has very similar 11–zinc finger domains to CTCF
  - can bind potentially bind to CTCF binding sites

- Remainder of protein is completely different than CTCF

- Expression is restricted to spermatogonia and pre–meiotic spermatocytes when imprints are being set up.

- BORIS is expressed in many cancer cell lines along with CTCF

- Ectopic expression of BORIS in cancer cells may disrupt CTCF function
Loss of imprinting of the Igf2/H19 genes by ectopic expression of BORIS

Displacement of CTCF by BORIS?

biallelic \textit{IGF2}
Overexpression of Igf2 via LOI contributes to tumorigenesis
LOI at *Igf2* results in a 2-fold higher incidence of intestinal adenomas in Min mice

When ICR deletion is inherited from the mother, the offspring have 2 active alleles of *Igf2*

<table>
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<th></th>
<th>no. of mice</th>
<th>avg no. of adenomas in intestine</th>
<th>fold increase</th>
<th>avg no. of adenomas in colon</th>
<th>fold increase</th>
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<td>81</td>
<td>28</td>
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<td>1.3</td>
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<td><em>Igf2</em>, paternal only</td>
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<td>2.2 p&lt;0.00001</td>
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<tr>
<td><em>Apc</em>+/− <em>H19-ICR</em>+/+</td>
<td>59</td>
<td>60</td>
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<td>2.9</td>
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Loss of imprinting (LOI) of IGF2 in tumors

- Wilms tumor
- Adrenocortical carcinoma
- Rhabdomysarcoma
- Hepatoblastoma
- Hepatocellular carcinoma
- Breast cancer
- Lung cancer
- Colorectal cancer
- Leiomyosarcoma
- Testicular germ cell cancer
- Renal cell carcinoma
- Kidney clear cell carcinoma
- Choriocarcinoma
- Cervical carcinoma
- Oesophageal cancer
- AML

- Many tumors have LOI of the *IGF2* gene
- This results in two active copies of *IGF2* and possibly the silencing of one or more maternally expressed 11p15 imprinted genes.
Other genes showing LOI in cancer

- **PEG1** in lung cancer
- **TP73** in gastric cancer
- **CDKN1C** in pancreatic cancer (loss of expression)
Cdkn1c

Cell cycle inhibitor (growth suppressor/TSG), normally expressed exclusively from the maternal allele
Overgrowth and cancer predisposition syndrome—1000-fold increased risk to specific embryonal tumors (i.e. Wilms’ tumor)

Imbalance in the expression of one or more 11p15.5 imprinted genes (contains both the IGF2/H19 cluster and the KvDMR1 cluster)

Main causes: Overactivity of IGF2 / loss of CDKN1C expression

>50% of cases due to abnormal DNAme
BWS Clinical Presentation

Omphalocele

Macroglossia

BWS and normal kidneys

BWS and normal pancreas

Ear Creases

Wilm’s tumor
BWS Molecular Subtypes

- **15% heritable (autosomal dominant)**
  - Inherited point mutations of Cdkn1c
    - Loss of a growth suppressor → overgrowth

- **85% sporadic**
  - 5% to 10% – point mutation at Cdkn1c
    - Loss of growth suppressor → overgrowth
  - 10% to 20% – Paternal UPD chromosome 11
    - Overexpression of IGF2 and “loss of expression” of CDKN1C and other maternally-expressed genes
  - 2% to 7% – Hypermethylation of H19 ICR
    - Higher risk of cancer
  - Rare – microdeletion of ICR
  - 50% to 60% – Hypomethylation of KvDMR1
    - Higher risk of abdominal wall defects and overgrowth
Kvdmr1 Cluster

- Kcnq1ot1 IncRNA expression from paternal allele silences genes in cis
- Methylation on maternal allele represses Kcnq1ot1 which allows expression of genes in cis
LOM at KvDMR1 and BWS

**Normal**

- m: PHLDA2 → SLC22A18 → CDKN1C
- p: PHLDA2 → SLC22A18 → CDKN1C

**BWS**

- m: PHLDA2 → SLC22A18 → CDKN1C
- p: PHLDA2 → SLC22A18 → CDKN1C

LOM of KvDMR1 → “activation” of KvDMR1 repressive function which prevents the expression of CDKN1C and other maternally-expressed genes.
Imprinted genes are expressed monoallelically, meaning that they are expressed from only one allele. In contrast to a majority of the genome, which is expressed biallelically.

In genomic imprinting, the way a gene is expressed is dependent on who’s chromosome it was inherited from (Mom’s or Dad’s), the sex of the offspring does not matter.

- Some imprinted genes are expressed from the maternal allele and repressed on the paternal.
- Some imprinted genes are expressed from paternal allele and repressed on maternal allele.

Imprints are erased and reestablished in the germ cells during development, this is important because sperm need to pass on certain imprints and eggs need to pass on different ones to their future offspring.
DNA methylation is the mark found on one of the parental alleles in areas termed differentially methylated regions (DMRs).

Sequence features, Specific chromatin signature at DMRs and transcription through DMRs may all play an important role in targeting imprinted genes for methylation.

Imprint Control Regions (ICRs) are found at DMRs and you may see the 2 terms used interchangeably in the literature. If a region is truly an ICR, deletion should result in LOI of one or more genes in cis. 2 examples of ICRs: H19 DMR & KvDMR1.

ICRs are often associated with insulators or long ncRNAs.

The H19/Igf2 cluster and the KvDMR1 cluster are both located on human chr 11p15.5 (distal mouse chr7).

BWS is an imprinting disorder characterized by overgrowth and cancer predisposition. Overexpression of Igf2 and loss of expression of Cdkn1c are often responsible for the BWS phenotype. Loss of Methylation at KvDMR1 accounts for >50% of cases of BWS.
Igf2 is a potent fetal mitogen and survival factor, it is normally expressed exclusively from the paternal allele, but in many cancers and BWS imprinting of this gene is lost and 2 copies are now expressed. LOI may result from PAT UPD, hypermethylation of the H19 ICR, BORIS displacement of CTCF, etc.

Cdkn1c is a cell cycle inhibitor (growth suppressor), it is normally expressed exclusively from the maternal allele, but in certain cancers and BWS, LOI leads to complete loss of expression. LOI may result from point mutations, PAT UPD, LOM at KvDMR, etc.
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Helpful Reading

TIMELINE

Genomic imprinting: the emergence of an epigenetic paradigm

Anne C. Ferguson-Smith

Mammalian Genomic Imprinting

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Nature Reviews: Genetics: 2011
Practice Questions

- Define Genomic Imprinting
- What is UPD?
- 2 main mechanisms of regulation in imprinted clusters, how they work and an example of a cluster that employs them and an example of how they might be deregulated.
- What is the significance of imprint erasure in the developing embryo?
- What may play an important role in targeting imprinted genes for methylation?
- What is an ICR?
- 2 Main genes involved in etiology of BWS, how is their expression changed from normal? Most common molecular subgroup.
- Mechanism by which Igf2 may lose imprinting.
- What is the relationship between CTCF and BORIS with respect to the Igf2/H19 cluster? (i.e. How would aberrant expression of BORIS affect CTCF and imprinting in the cluster?)