Changes in the pattern of Imprinted Genes expression in Pediatric Tumors

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Background

- Cancer is the **first-leading cause** of death from disease in children!
- **New interventions** are needed to prevent recurrences, extend survival, and cure many types of pediatric cancer.
- **Imprinting genes** play a role in cancer propagation - shown Loss Of imprinting in Adult Tumors
- In previous studies in our laboratory a Link between Normal and Tumor Progenitor Cells of the Developing Human Kidney was demonstrated.

Aims

- To identify **changes in the pattern of imprinted genes expression** along tumor progression in XN models of pediatric tumors
- Better understanding the mechanisms of imprinting and LOI will help in discovery of new molecular targets. This **Epigenetic** process may be reversible, and enable targeted therapy Interventions.

Methods

- **PDX model** – In order to increase the number of tissues.
- **Microarray** of the imprinted genes and find significant difference in genes expression
- **RNA seq** – Originally we planned to perform RNA-seq in order to evaluate the significant of the difference in gene expression, but it wasn't done because of unsatisfactory quality of the RNA extracted from tissues
- **Bisulfide conversion** – To check if the changes in gene expression is connected to methylethion status (LOI).

Bisulfide conversion

- **Differentiate between methylated and unmethylated cytosine residues**.

- Unmethylated cytosine residues are deaminated to uracil and methylated cytosine (5-mC) residues remain unaffected.
- Enabling PCR amplification to recognize uracils as thymines and 5-mC or 5-hmC as cytosines.

Template:
(Double Stranded)

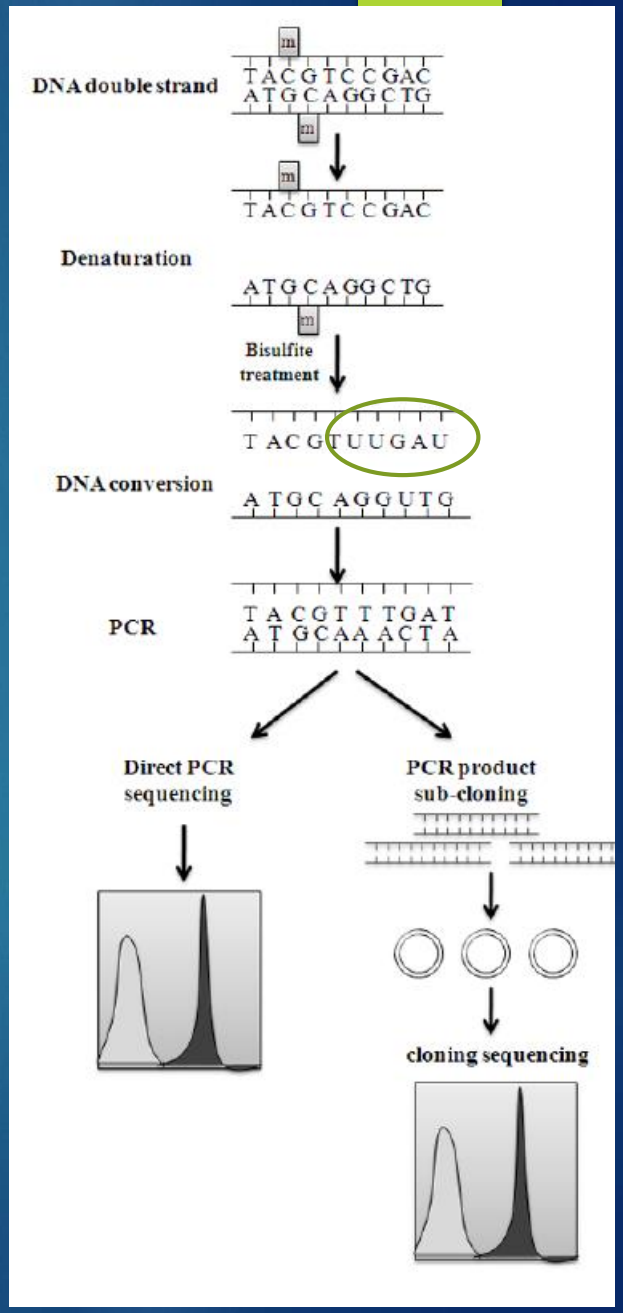
A: 5'-GACCGTTCCAGGTCCAGCAGTGCGCT-3'

B: 3'-CTGGCAAGGTCCAGGTCGTCACGCGA-5'

Bisulfite Converted:
(Single Stranded)

A: 5'-GATCGTTTTAGGTTTAGTAGTGCGTT-3'

B: 3'-TTGGCAAGGTTTAGGTTGTTATGCGA-5'



Study features

- **Samples**:
  - Primary tumor tissue
  - Adult healthy tissue
  - Fetal healthy tissue
  - Transfered Xenograft
- **Population** : 5 types of Pediatrics Tumors
  - PPB
  - MRT
  - EWING sarcoma
  - Wilms
  - Medulloblastoma
- **Measured Fold changes:** Significant changes of gene expression defined as >2 or < 1/2 fold change. Meaning that the probability of these changes in healthy tissues are little.
- **No bias** in research plan, these tumors are really rare

Study Limitations

- **Concluding** from 1 tissues (of every tumor) to all this cancer type
- **Study size:** only 6 tissues not enough data for statistical analysis.
- **External validation:** will be performed at later stages by collecting other tumors and checking specific genes.
- **Internal validation**: no ability to validate the results although we used in strong bioinformatics tools.

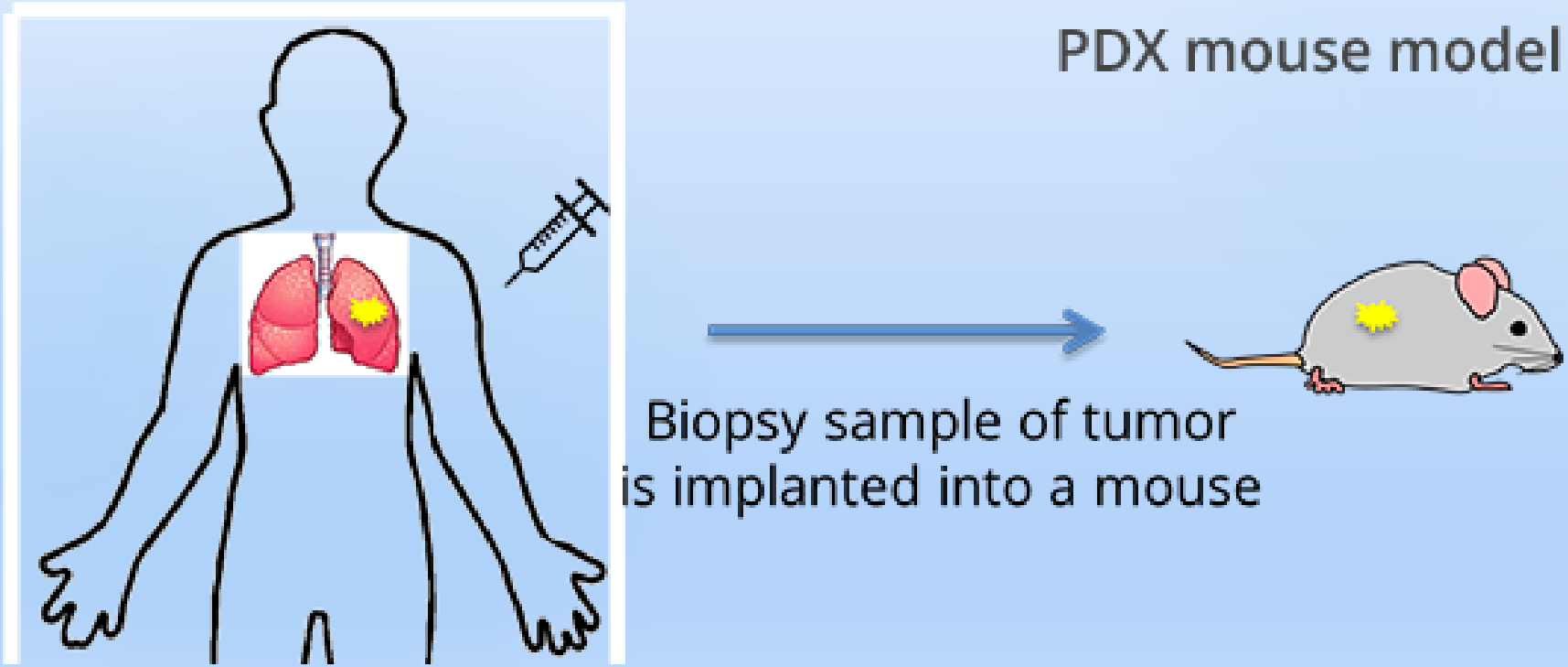
Results and Future Tasks

- We **Identified 15 genes** which have shown significant changes in expression
- **Validation** that the cause is connected to methylation status using Bisulfide conversation.
- **Understanding** and finding the role of the genes in the cancer system.
- If possible, **get other tissues** for control samples (**TCGA** The Cancer Genome Atlas ,a landmark cancer genomic program)

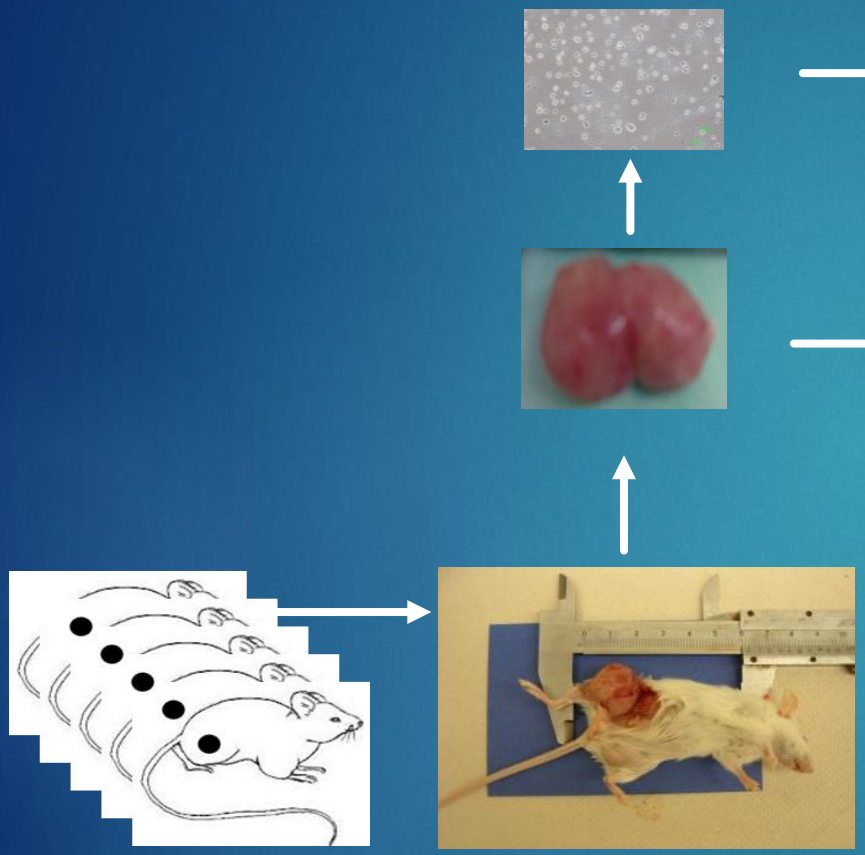
Analyzing Data and comparisons between tissues

I	H	G	F	E	D	C	B	A
AB VS FB	RTK vs AB	RTK vs ES	RTK vs FB	P17 VS PT	PT VS AB	PT vs ES	PT VS FB	שם הגן
X	+	-	+	-	+	-	+	AIM1
X	-	X	-	X	-	X	-	BLCAP
X	-	X	-	X	-	X	-	DLGAP2
X	-	X	-	X	-	X	-	GNAS
X	-	-	X	X	-	-	X	GRB10
X	-	-	-	X	-	-	-	INPP5F
X	X	X	X	-	X	X	X	KCNQ1
+	++	++	++	X	++	++	++	**MEST**
X	++	++	++	++	++	++	+	PLAGL1
X	X	X	+	X	X	X	+	L3MBTL1
+	-	-	-	-	-	-	+	PPP1R9A
X	X	--	-	X	X	--	-	SNRPN /// SNURF
X	X	X	X	X	X	X	+	UBE3A
X	X	X	X	X	X	X	X	ZNF331
++	-	X	+	X	+	++	++	WIF1
X	-	X	-	+	-	X	-	DIRSA3
X	++	++	++	-	++	++	++	IGF2 /// INS-IGF2
+	X	+	+	X	+	X	+	**PEG3**
X	-	+	-	X	X	+	X	FAM50B
X	-	-	-	X	X	X	X	MAGEL2
x	+	x	+	x	+	X	+	MCTS1 /// PSIMCT-1
x	--	x	--	x	-	+	-	MKRN3
X	-	X	-	X	-	X	-	LRRTM1
X	X	X	X	+	X	X	X	DLK1

A platform to discover therapeutic cancer stem cell antigens in rare pediatric tumors using patient derived xenografts (PDX)

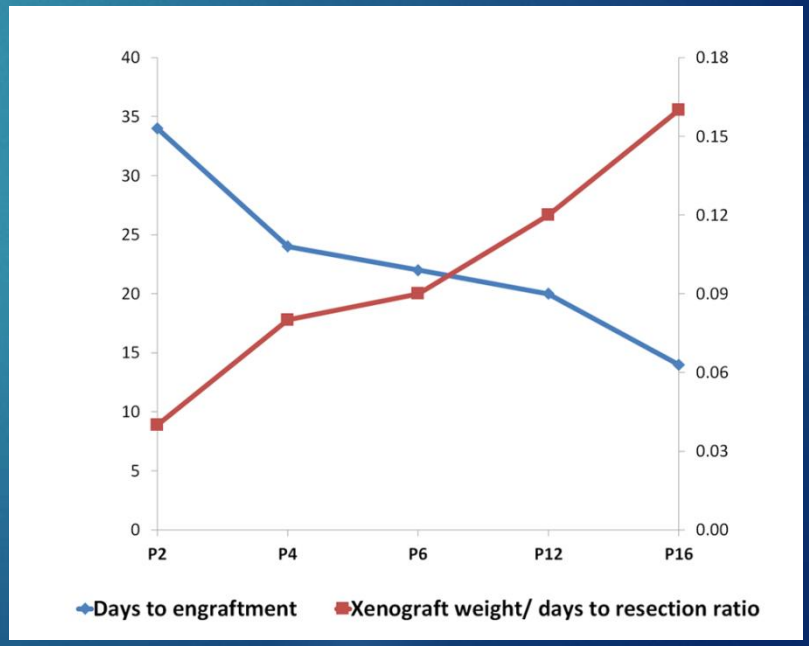


During serial propagation of PDX shorter time to tumor engraftment , accelerated tumor growth and less amount of cells needed to establish Xn were demonstrated - indicating the promotion of tumor aggressiveness and stemness along passages



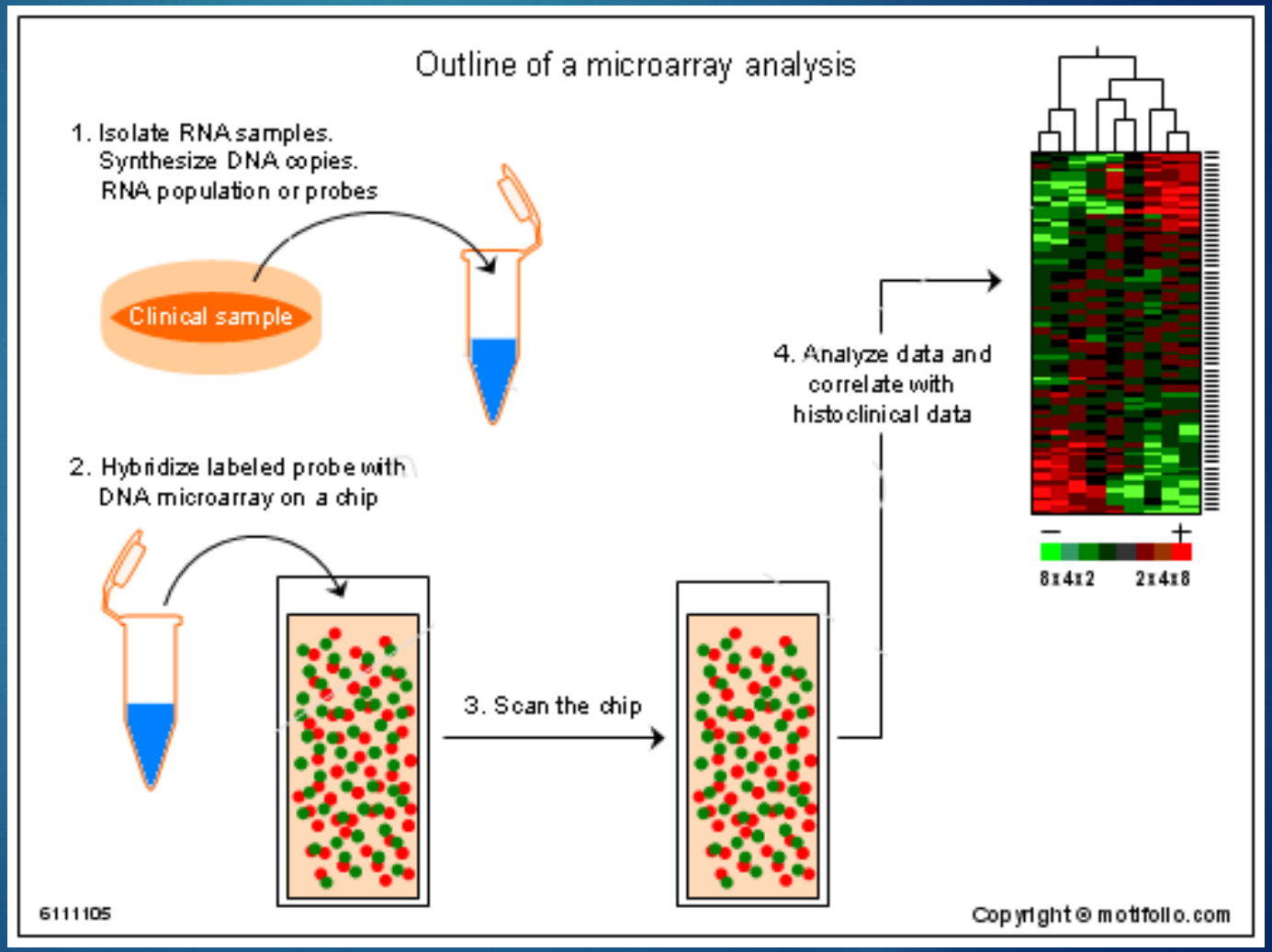
1x10⁶ cells

50 cells



Characterization of the molecular profile accompanying the selection for the CSC phenotype during Xn propagation

- Primary tumor
- Passage 2
- Passage 4
- Passage 6
- Passage 8
- Passage 12
- Fetal Tissue
- Adult Tissue



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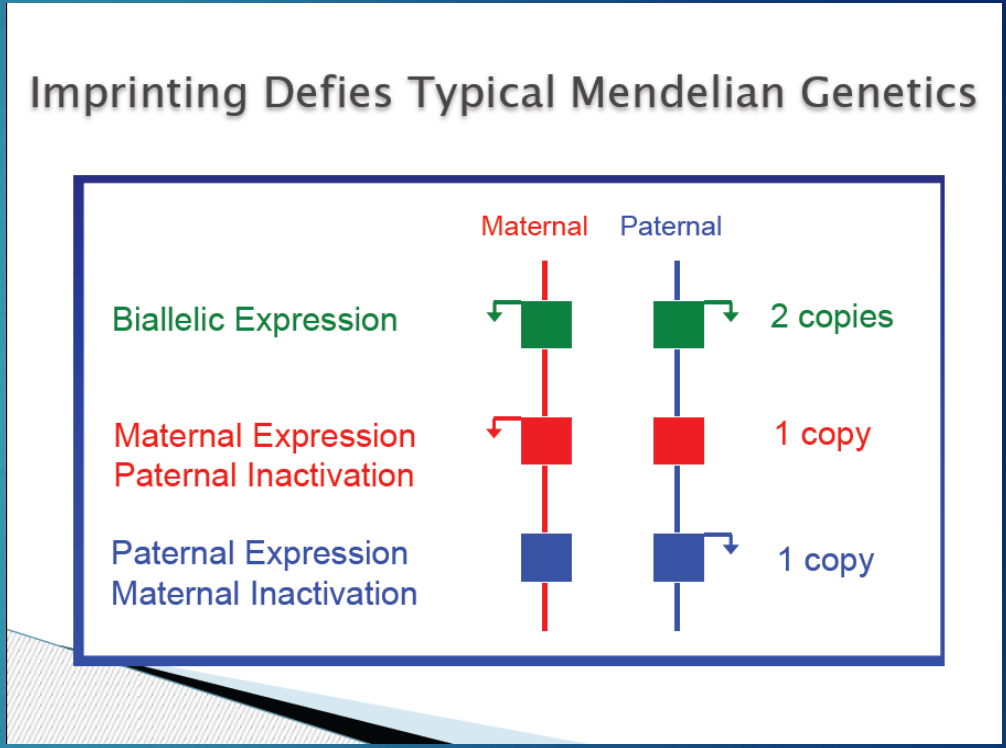
Imprinted genes

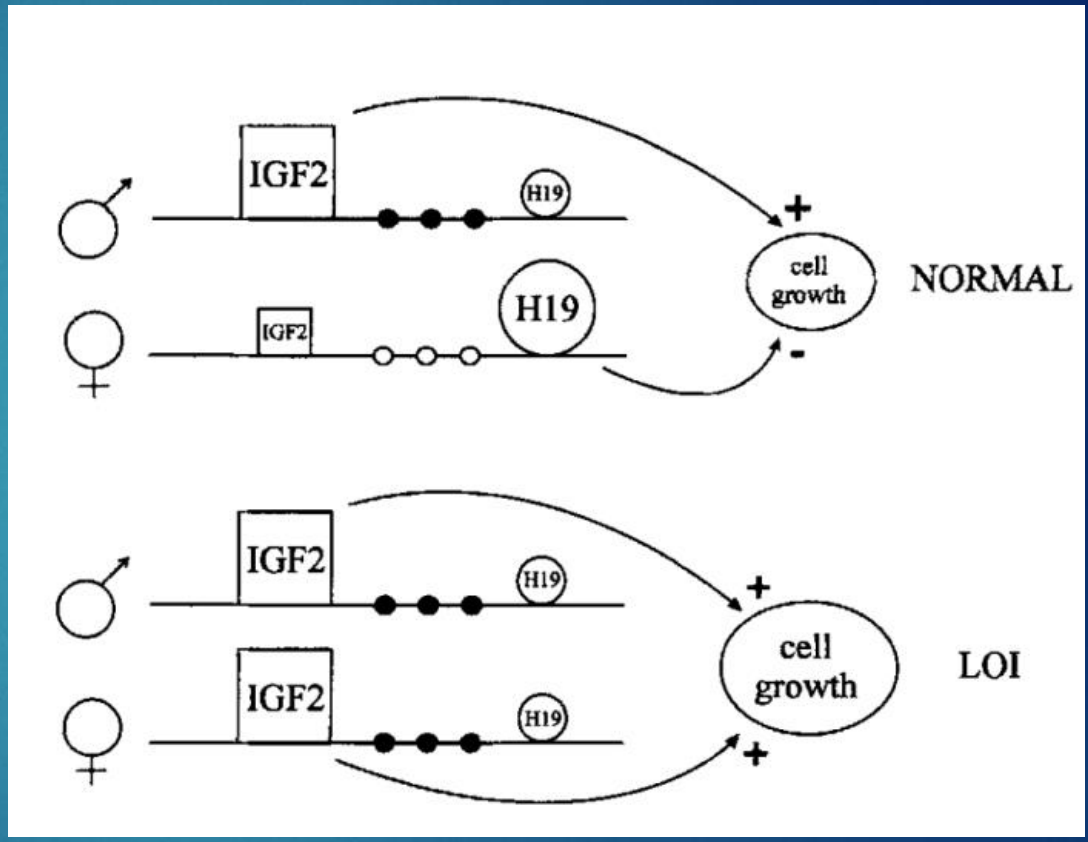
- **Monoallelic expression**
- Involved in growth regulation and development

- **The gene was epigenetically marked or imprinted in either the egg or the sperm**
- Imprinting status varies between tissues, developmental stages and species



Loss of imprinting

- **Disruption of the normal monoallelic expression pattern** of an imprinted gene.
- **complete (biallelic) silencing or biallelic transcription** that is thought to cause higher expression levels, mainly by alterations in DNA methylation at iDMRs.



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