Modulation of the epigenome in fish carcinogenesis

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Gothenburg

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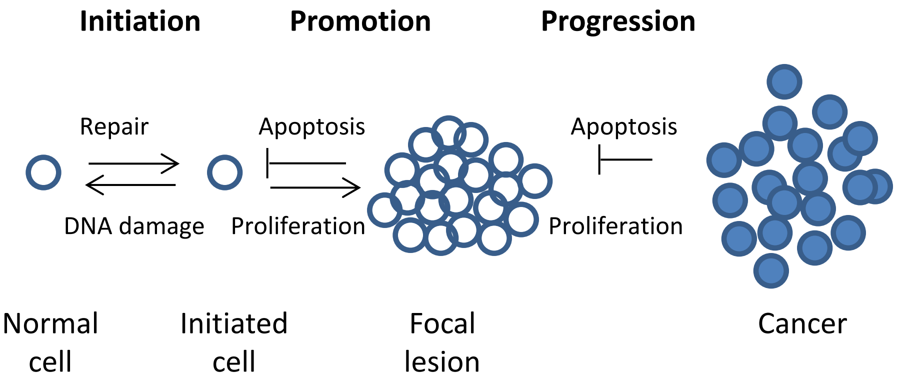
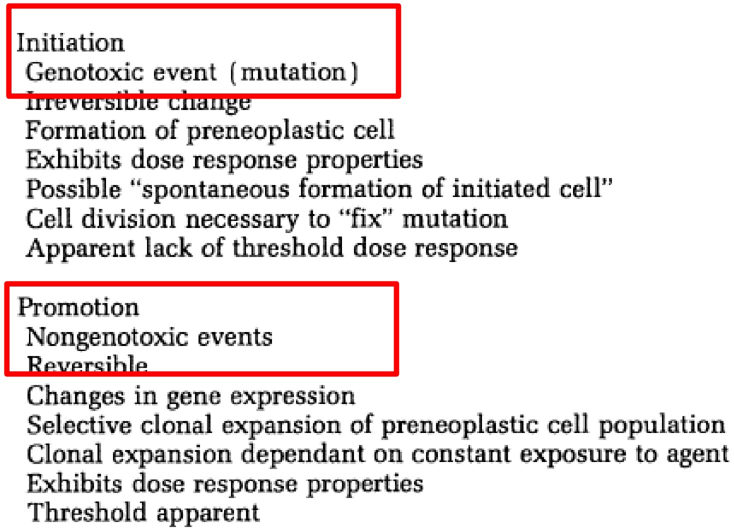
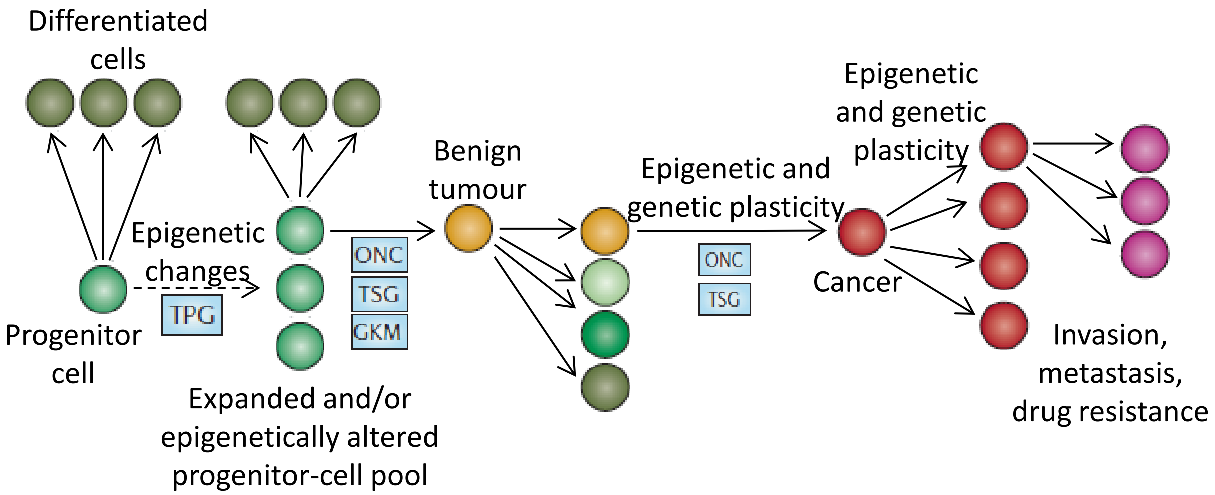
Genome–epigenome interactions in cancer



Multistage process of carcinogenesis.
C Nordling 1953; Knudson 1971; Klaunig 2000


Initiation
Genotoxic event (mutation)
Irreversible change
Formation of preneoplastic cell
Exhibits dose response properties
Possible "spontaneous formation of initiated cell"
Cell division necessary to "fix" mutation
Apparent lack of threshold dose response

Promotion
Nongenotoxic events
Reversible
Changes in gene expression
Selective clonal expansion of preneoplastic cell population
Clonal expansion dependant on constant exposure to agent
Exhibits dose response properties
Threshold apparent

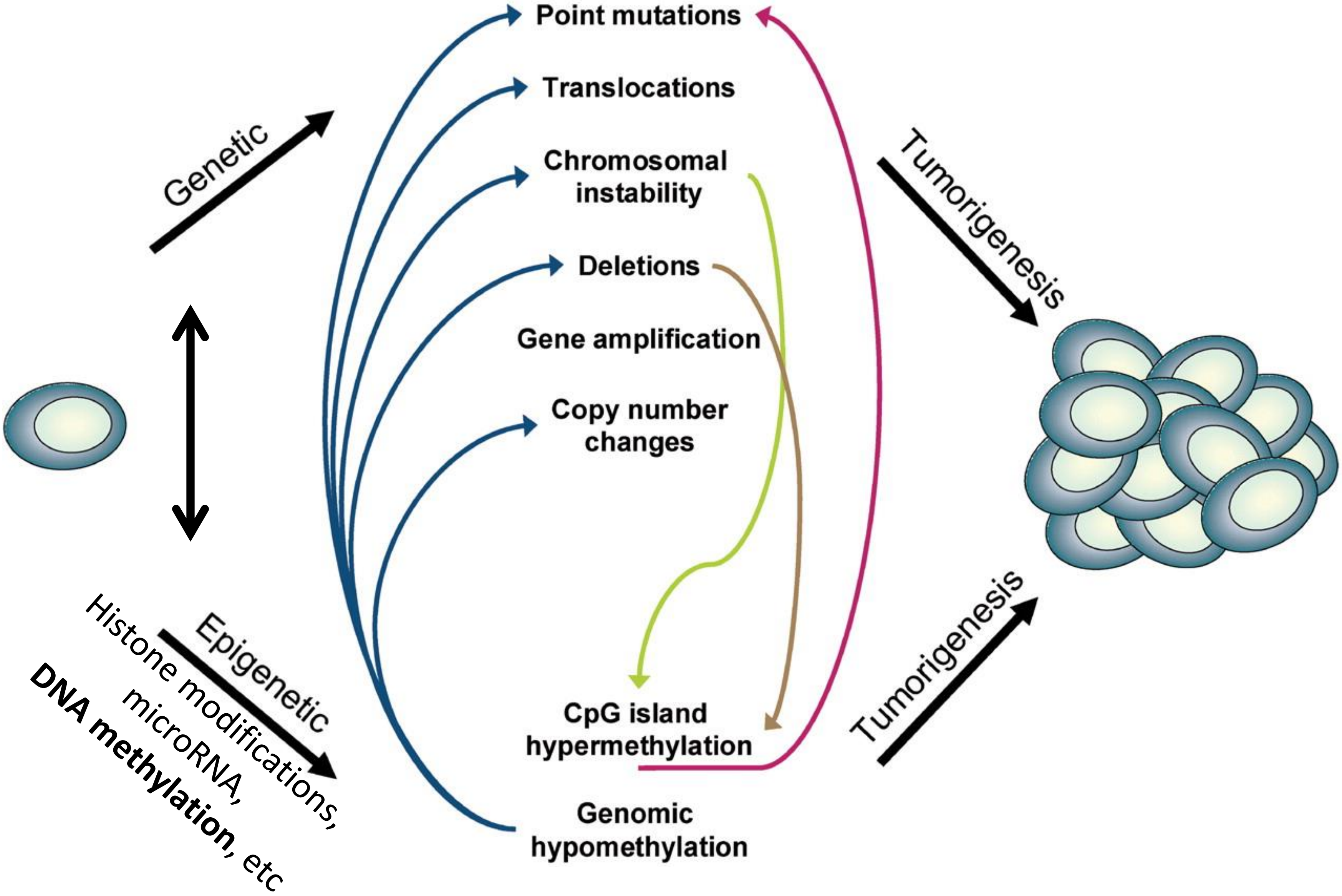


Epigenetic progenitor model of tumourigenesis. Feinberg et al., 2006

The first step involves an epigenetic disruption of progenitor cells in a given organ or system, which leads to a polyclonal precursor population of neoplasia-ready cells.

cancer has both a genetic and epigenetic basis

Genome–epigenome interactions in cancer



Genetic

Epigenetic

Histone modifications,
microRNA,
DNA methylation, etc

Point mutations

Translocations

Chromosomal instability

Deletions

Gene amplification

Copy number changes

CpG island hypermethylation

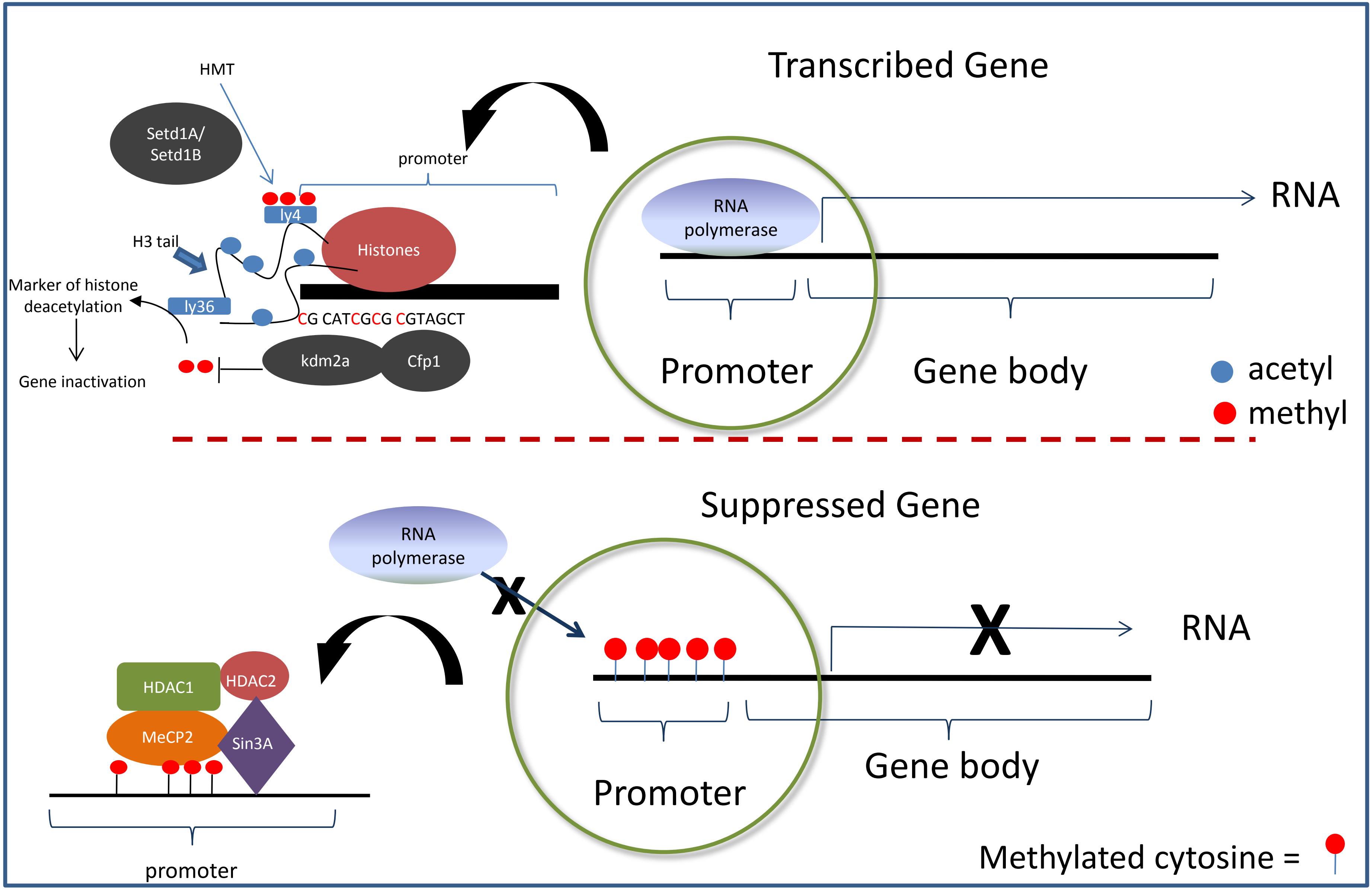
Genomic hypomethylation

Tumorigenesis

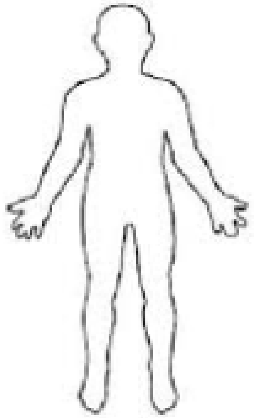
Tumorigenesis

Modified from Brena R M , and Costello J F Hum. Mol. Genet. 2007;16:R96-R105

DNA methylation and regulation of gene expression



Why study epigenetics in other non-mammalian species such as fish?

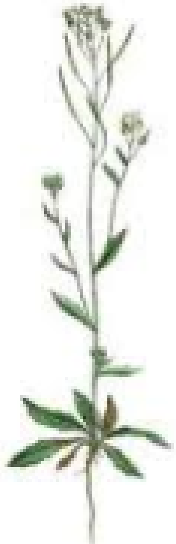


Human cell lines and tissues

Mice and rats

bees

earth worm



plants

Daphnia

Danio rerio

Flatfish

Images not to scale!

Why study epigenetics in other species such as fish?

It has been shown that environmental exposure to chemicals can modulate epigenetic marks in environmentally relevant species such as fish.

Three-spine Stickleback

17b-oestradiol (E2: 100 ng/l, 22–23 days) significantly increases DNA methylation in male gonads

Aniagu et al. (2008) Environ Int 34(3):310–317.

Zebrafish

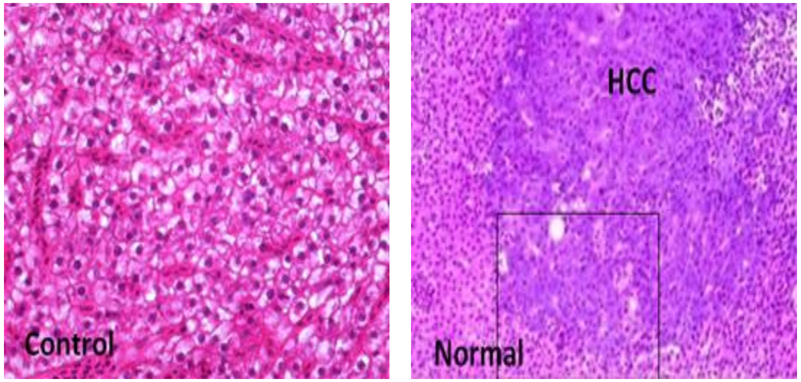
17a-ethynilestradiol (EE2: 100 ng/l, 14 days) significantly decreases the methylation levels of several CpG sites in the 5’ region of the vtg gene in the liver of males and females zebrafish

Stromqvist et al. (2010) Aquat Toxicol. 98(3):275–281.

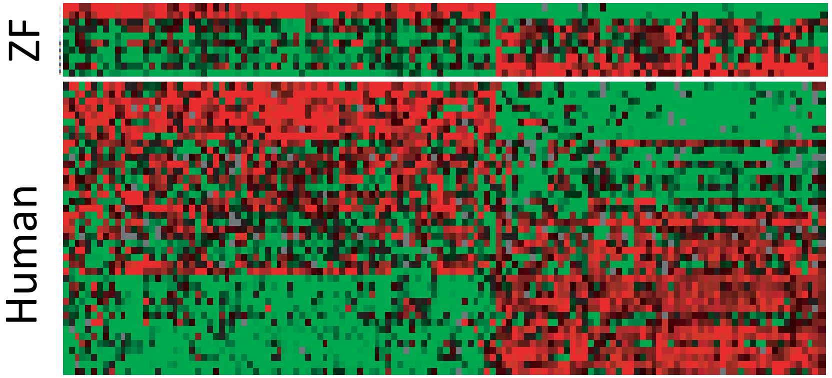
Changes in the epigenome can potentially have major consequences, such as resulting in development of **tumours**

Conservation of gene expression signatures between zebrafish and human liver tumours and tumour progression

Lam et al. Nature Biotechnology. 2006. 24: 73-75



Three week old zebrafish fry were treated with DMBA (0.75 ppm)

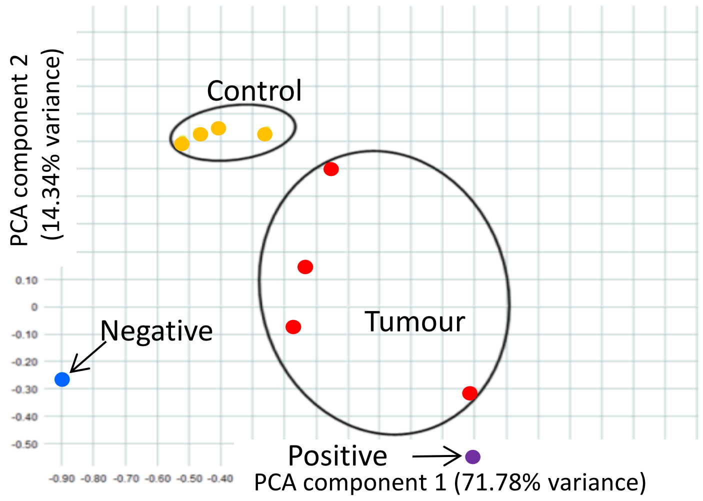


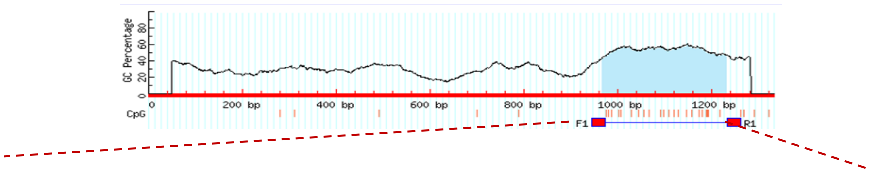
Expression profile of 132 genes showing similar correlation with tumour progression in both zebrafish and human liver tumours

What about the contribution of epigenetic mechanisms to development of liver tumours in zebrafish?

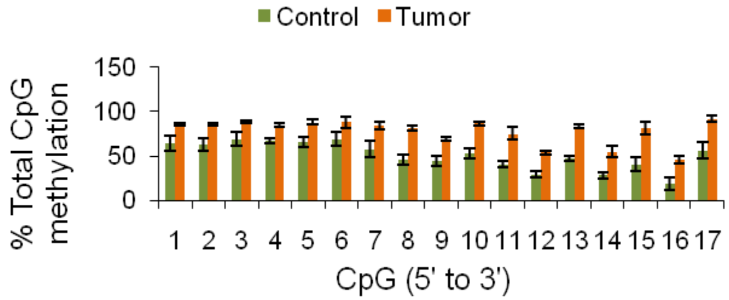
DNA methylation alterations in zebrafish liver tumours (Mirbahai et al. BMC Genomics. 2011. 12:3)

PCA scores plot of methylation profiles





CCGGAGCTCCAAGTTTCGAAACGTCTATGGCAAAGTGGCCAACCGAGAGCACTGCTTCGAGGGCATCCC
GATCACAAAGAACGTCCATGACAACCACTTCTGTGCGGTCAACGCCAAGTTCCTCGCCATAGTGACGGA
AAGCGCGGGAGGAGGATCTTTCGTTG



Biological categories of genes with altered methylation

The canonical pathway “molecular mechanisms of cancer” was enriched in HCC compared to Control

Hypermethylated (~ 200 regions)

- Anti-angiogenesis
- Cell-cell adhesion
- Oxidative stress protection

Hypomethylated (~ 700 regions)

- Glycolysis
- Proliferation
- Angiogenesis, metastasis, adhesion, cell growth, cell cycle and response to stress

Dab (*Limanda limanda*)

- Flatfish used in offshore biomonitoring programme

- High cancer prevalence (~20%) Irish sea & North sea

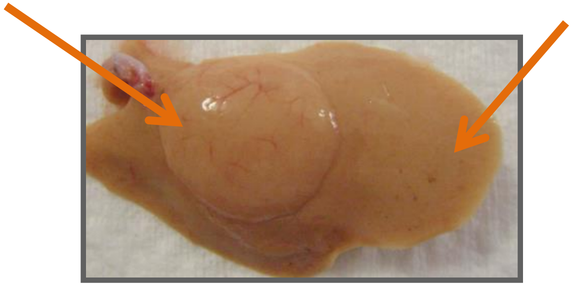
- Molecular basis of tumourigenesis is unclear-

---is modulation of DNA methylation contributing to changes in gene expression?

- Unsequenced

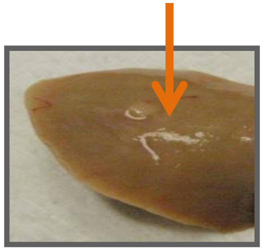
Three categories of samples were used to investigate the role of DNA methylation in dab tumours

1. HCA (T)



2. Histologically normal non-cancerous distal tissue (DT)

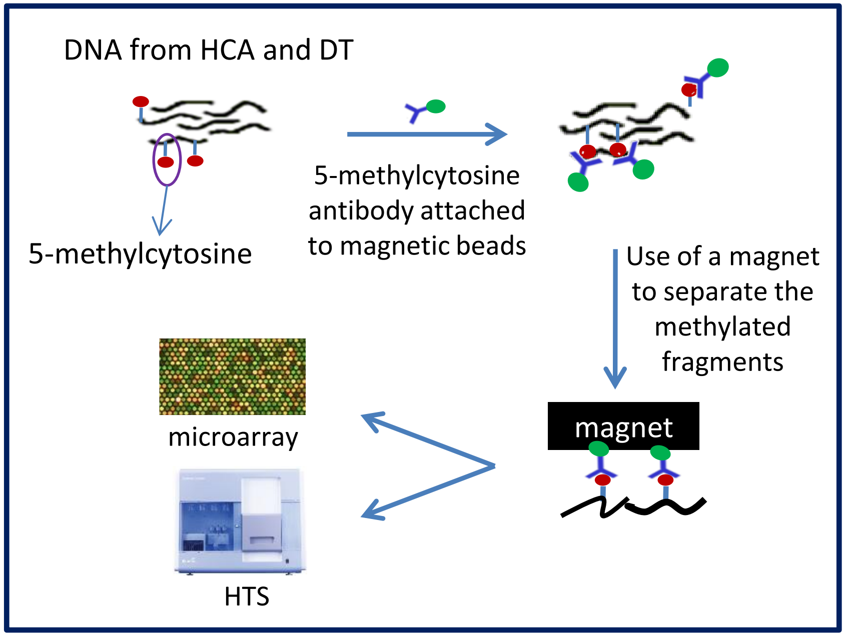
3. Healthy liver



Mirbahai et al. Epigenetics. 2011: 6 (11) 1319-1333.

DNA methylation alterations

- ✓ HPLC for Global DNA methylation measurements
- ✓ MeDIP coupled to microarray
- ✓ MeDIP coupled to high throughput sequencing
- ✓ Bisulfite sequencing PCR

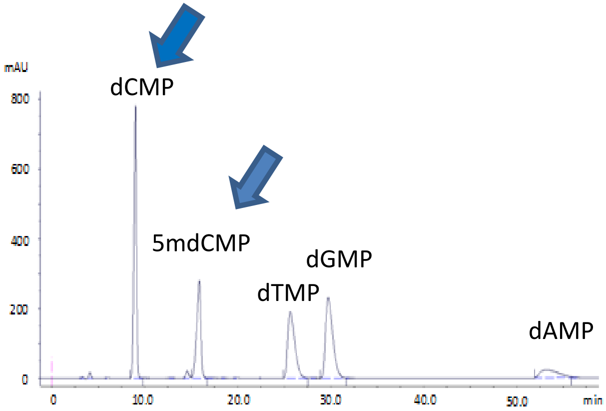


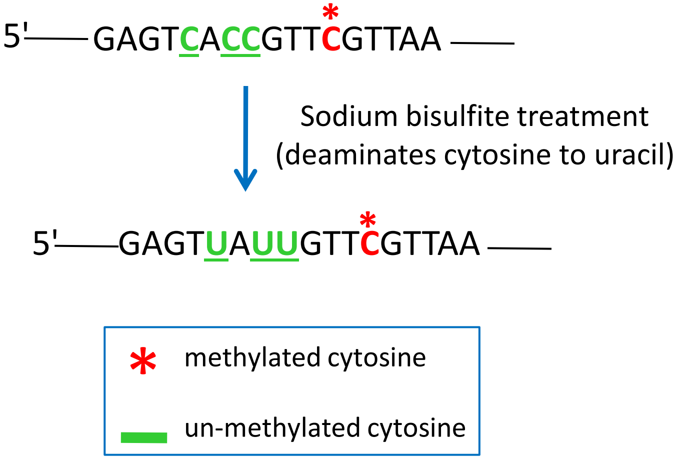
DNA from HCA and DT

5-methylcytosine

5-methylcytosine antibody attached to magnetic beads

Use of a magnet to separate the methylated fragments





5'—GAGTCACCGTT*CGTTAA —

Sodium bisulfite treatment (deaminates cytosine to uracil)

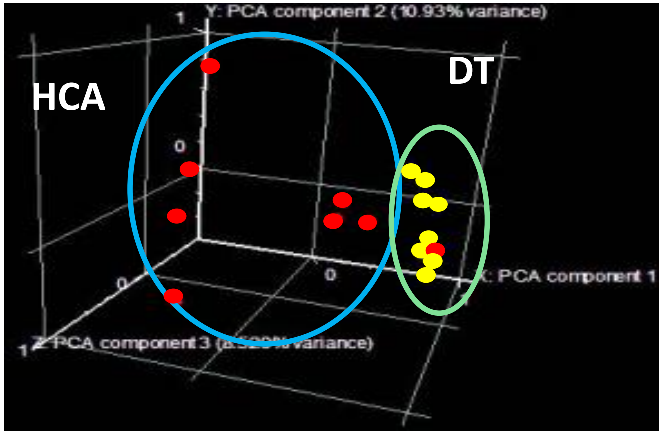
5'—GAGTUAUUGTT*CGTTAA —

*	methylated cytosine
🟩	un-methylated cytosine

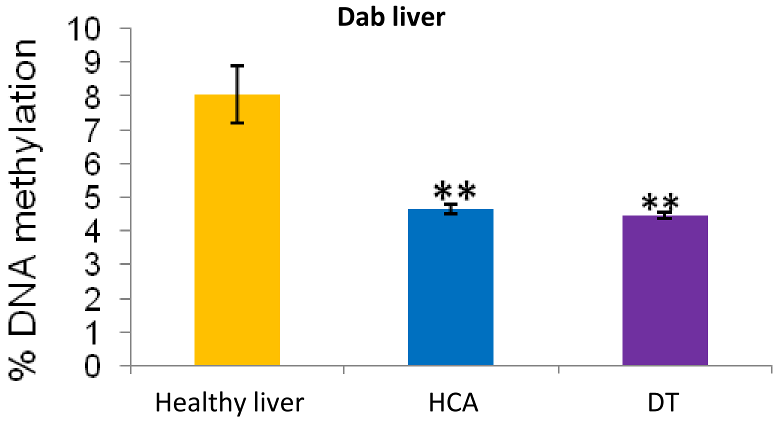
DNA methylation alterations

The genes identified with altered DNA methylation in HCA samples compared to DT have biological functions associated with general hallmarks of cancer such as:

- Cell death and cell cycle
- Cell to cell signalling and interaction
- DNA replication and repair

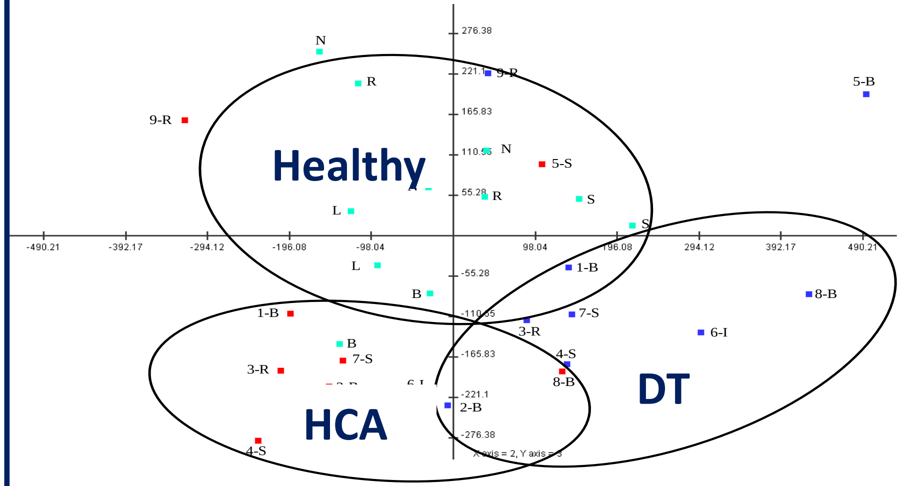


PCA on MeDIP-microarray data
(~1000 and 800 regions were hypo- and hyper-methylated respectively)

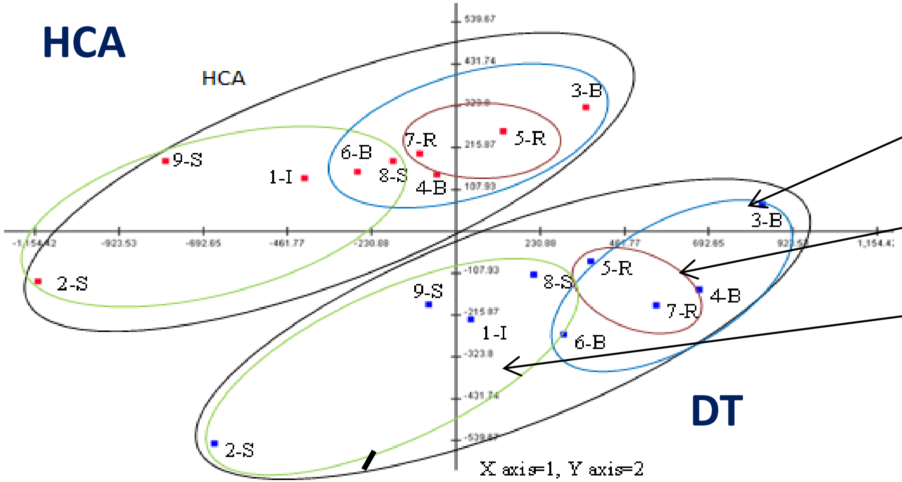


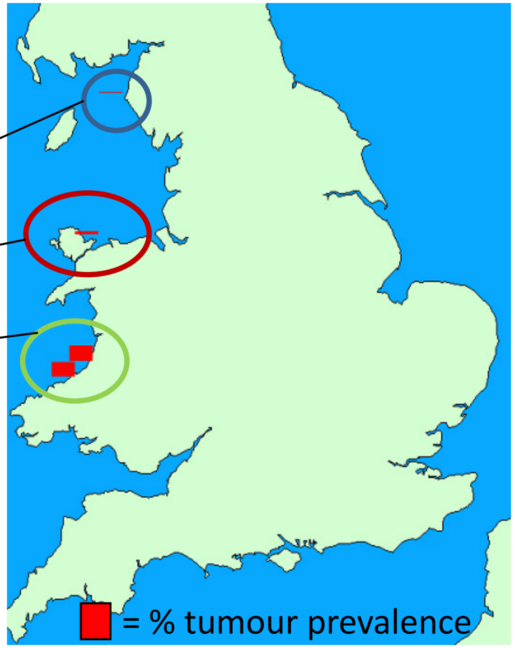
HPLC data

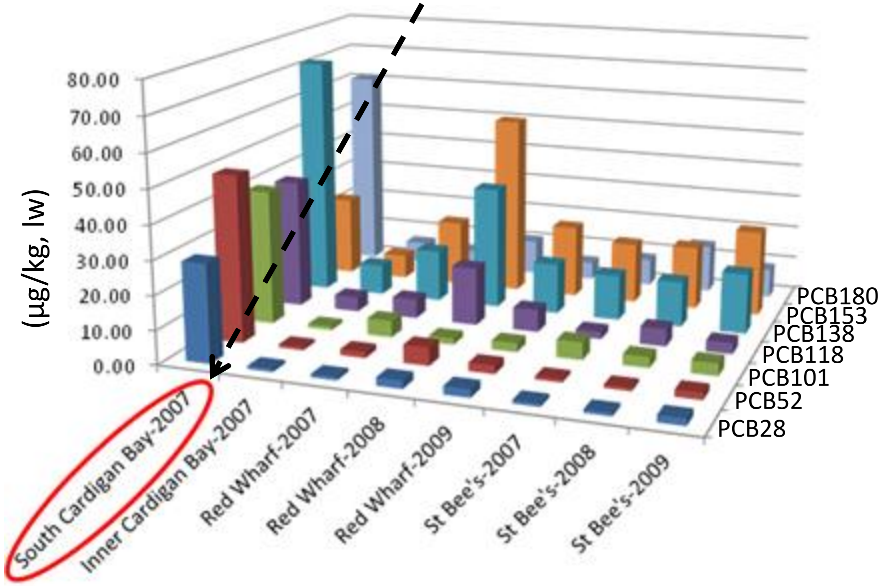
Alterations in gene expression



Tumour prevalence varies at different sampling sites. This shows that there is a link between environment (contamination level/type) and tumourigenesis which may be manifesting through epigenetic mechanisms





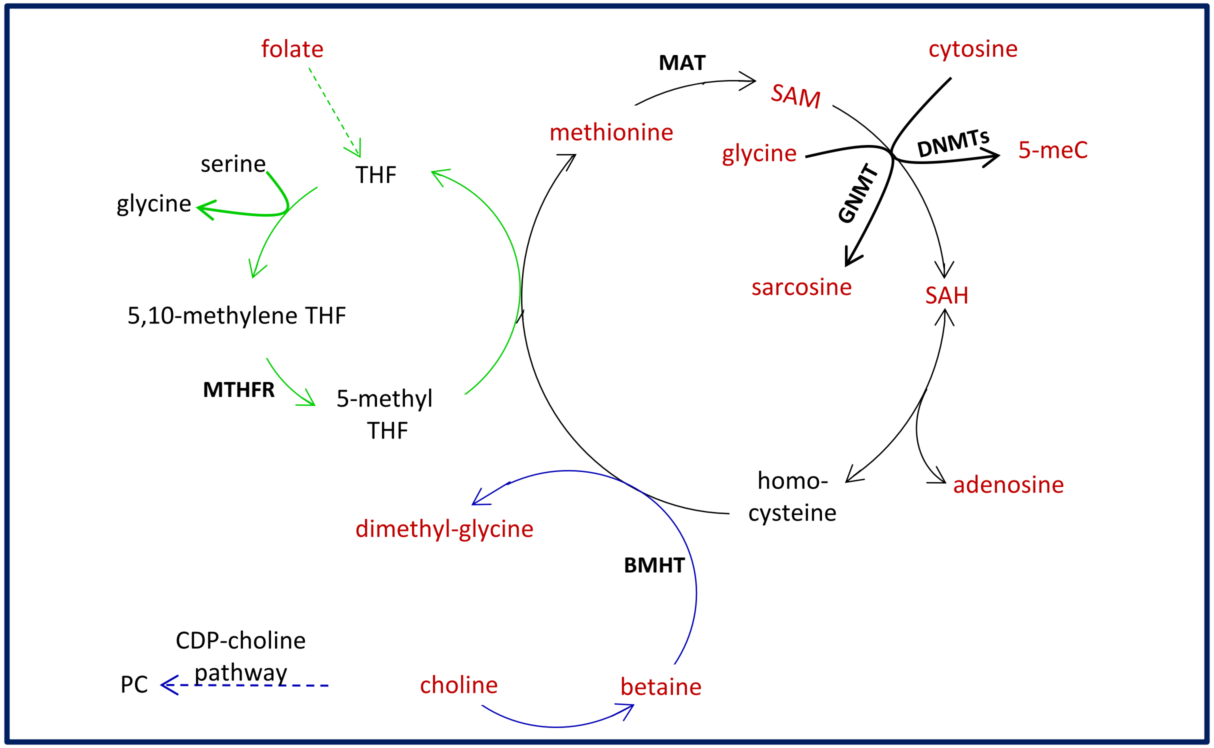


- PCBs have estrogenic and DNA methylation modifying properties

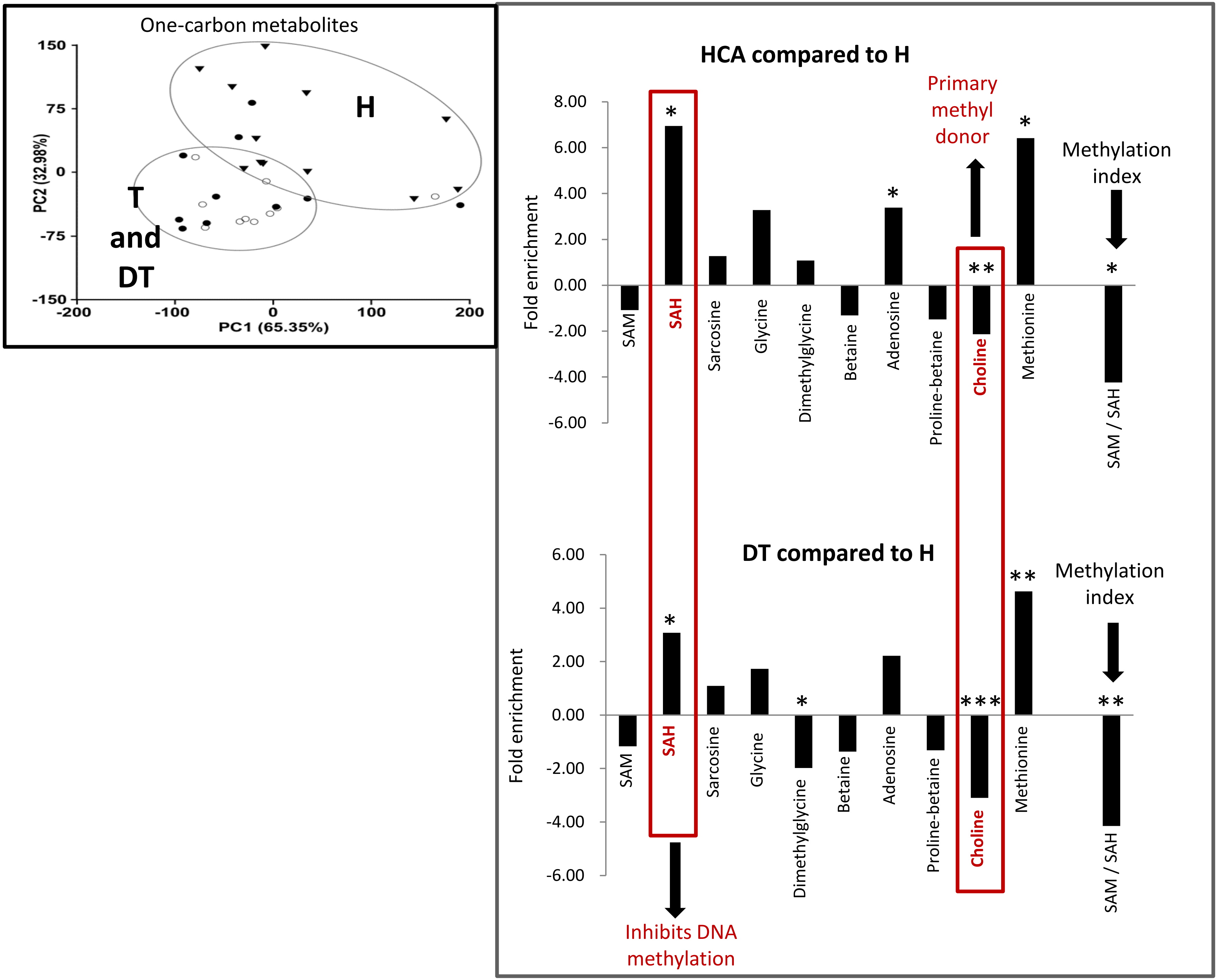
- Change in methylation and expression of vitellogenin gene (biomarker of exposure to estrogenic compounds) and other genes controlled by the estrogen receptors that are linked to tumourigenesis was observed.

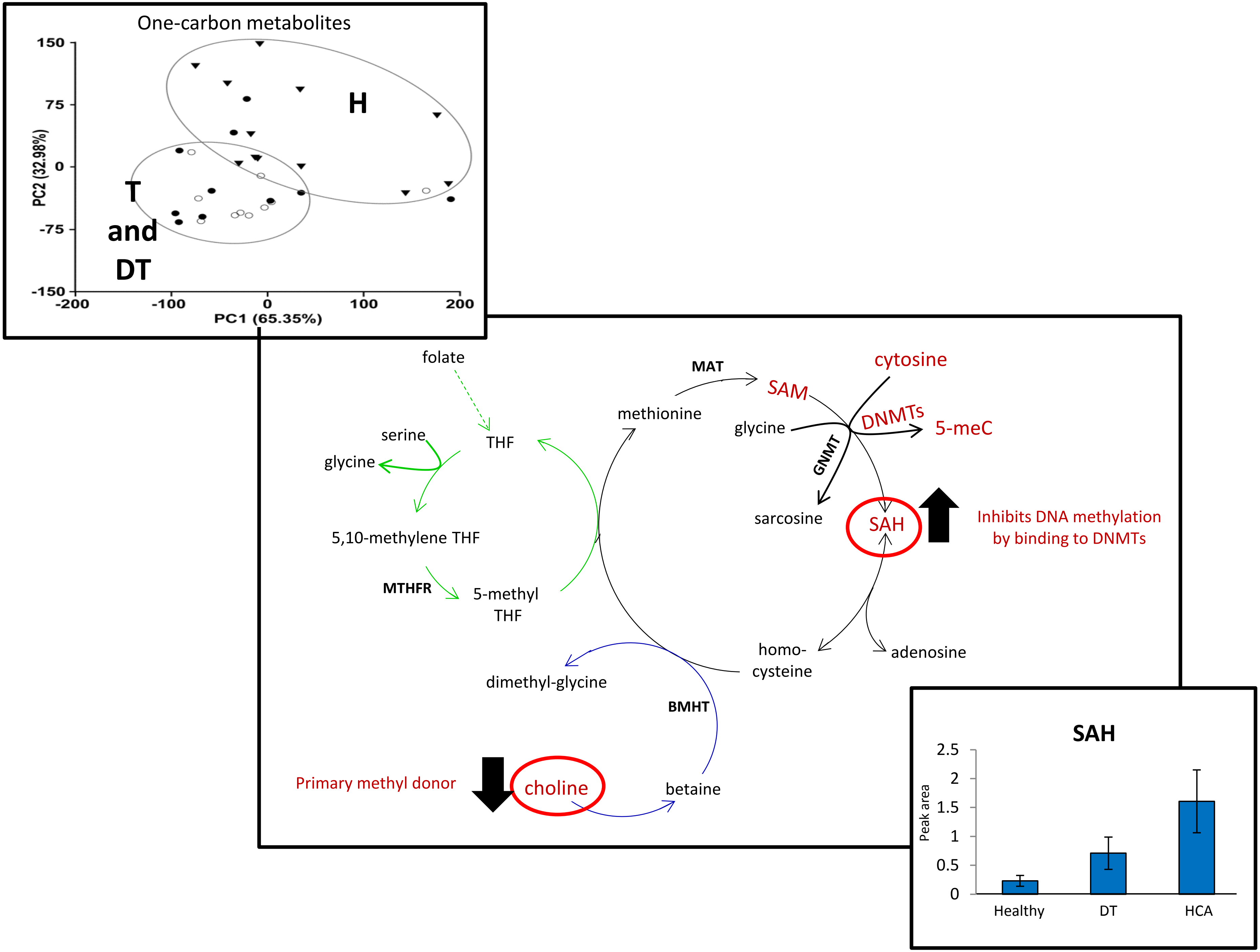
One Carbon pathway

- The principal biochemical pathway regulating DNA methylation
- Chronic imbalance in the concentrations of one-carbon cycle metabolites can influence DNA methylation and underlies the pathogenesis of many diseases
- LC-MS/MS was used to measure the concentration of the key metabolites in T, DT and H tissues



Mirbahai et al. J. Proteome Res., 2013: *12* (6) 2895–2904


One-carbon metabolites
150
75
0
-75
-150
PC2 (32.98%)
-200
-100
0
100
200
PC1 (65.35%)
H
T
and
DT
HCA compared to H
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
Fold enrichment
SAM
SAH
Sarcosine
Glycine
Dimethylglycine
Betaine
Adenosine
Proline-betaine
Choline
Methionine
SAM / SAH
*
*
*
**
*
Primary methyl donor
Methylation index
DT compared to H
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
Fold enrichment
SAM
SAH
Sarcosine
Glycine
Dimethylglycine
Betaine
Adenosine
Proline-betaine
Choline
Methionine
SAM / SAH
*
*
***
**
**
Methylation index
Inhibits DNA methylation


One-carbon metabolites
150
75
0
-75
-150
-200
-100
0
100
200
PC2 (32.98%)
PC1 (65.35%)
H
T
and
DT
folate
serine
glycine
THF
5,10-methylene THF
MTHFR
5-methyl
THF
dimethyl-glycine
BMHT
Primary methyl donor
choline
betaine
MAT
methionine
SAM
cytosine
glycine
DNMTs
5-meC
GNMT
sarcosine
SAH
Inhibits DNA methylation
by binding to DNMTs
homo-
cysteine
adenosine
SAH
Peak area
2.5
2
1.5
1
0.5
0
Healthy
DT
HCA

Overall findings

- Global DNA methylation level is altered in HCA and Distal Tissue (DT) in comparison to healthy liver

- The major metabolic differences were in HCA and DT compared to liver of non-tumour-bearing fish

- The mechanism of this disruption is linked to a decrease in choline (primary methyl donor) and elevated *S*-adenosylhomocysteine (SAH), a potent inhibitor of DNA methyltransferase.

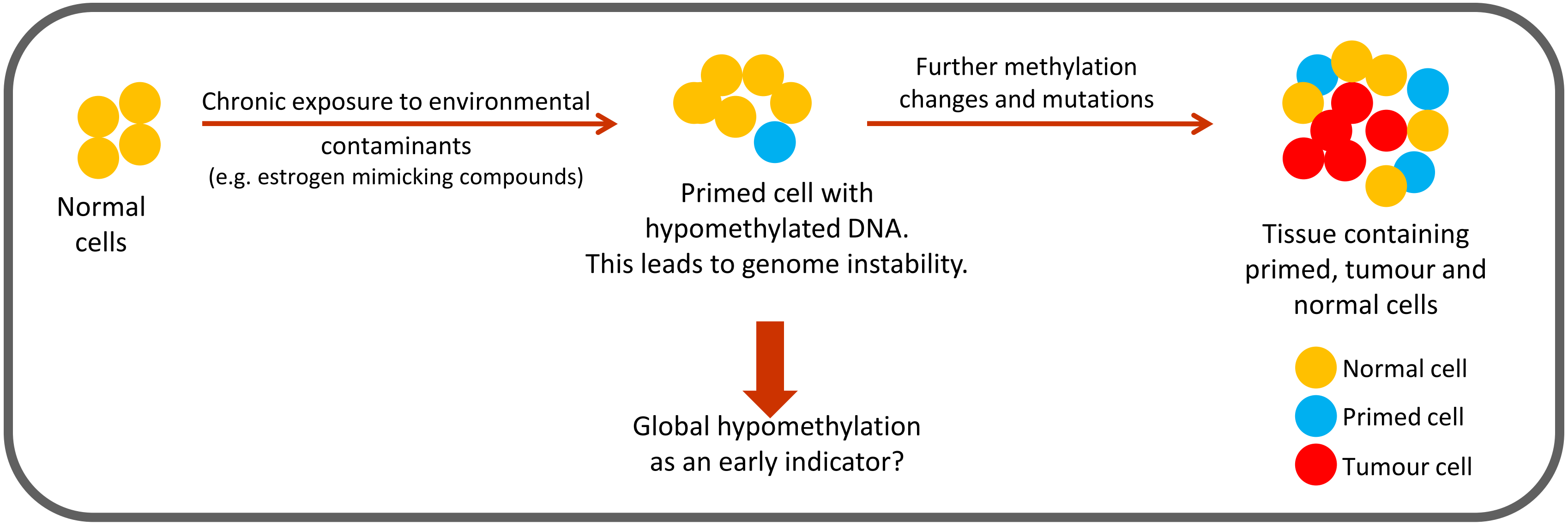
- The observed characteristics of DT (and the similarities with many features of the tumour cells) is particularly important since it highlights that using DT as a control in studies aiming to characterise differences between tumour and “healthy” tissue is not advisable

The finding of epigenetic modulation in Distal Tissue raises these intriguing questions:

1. is it a secondary response to the presence of tumour?

or

2. is it imposed in response to environmental conditions, predisposing the animals to carcinogenesis (epigenetic progenitor model of tumourigenesis)?



Normal cells

Chronic exposure to environmental contaminants (e.g. estrogen mimicking compounds)

Primed cell with hypomethylated DNA. This leads to genome instability.

Further methylation changes and mutations

Tissue containing primed, tumour and normal cells

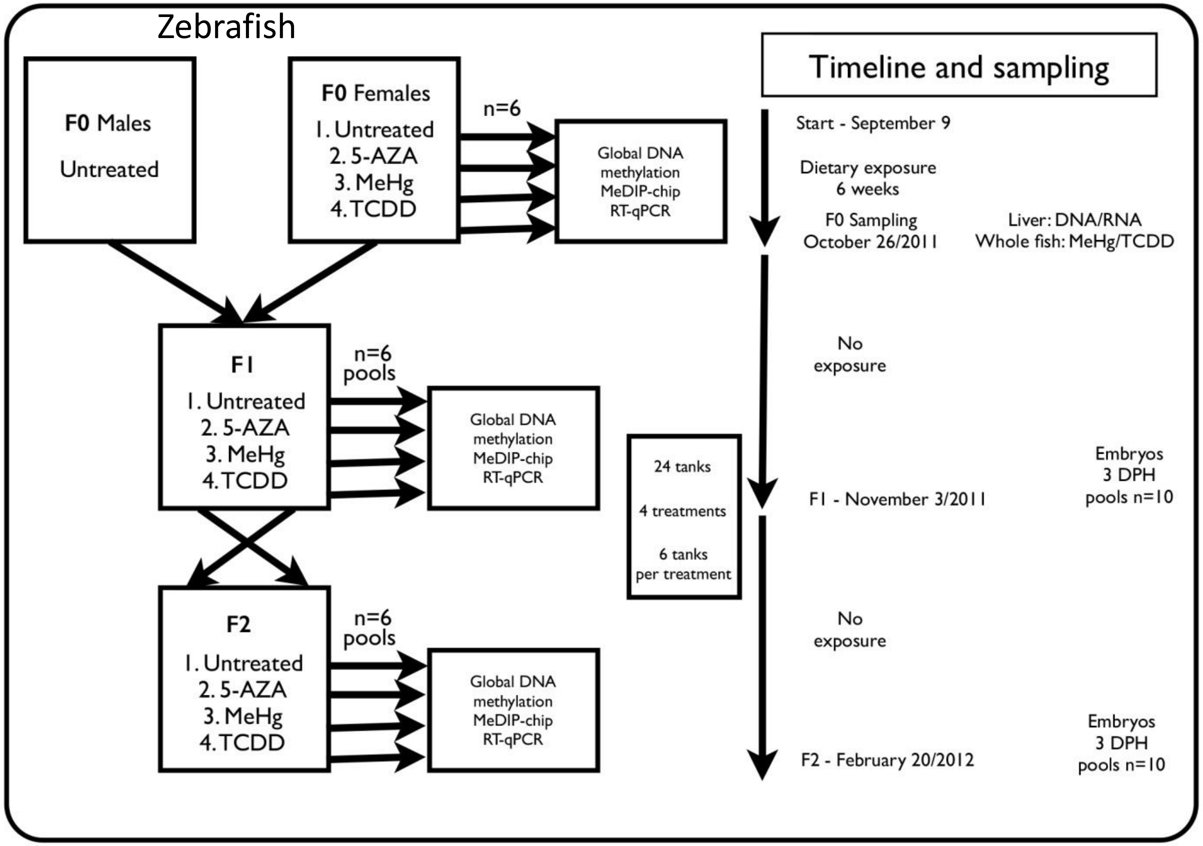
Global hypomethylation as an early indicator?

🟡 Normal cell
🔵 Primed cell
🔴 Tumour cell

“epigenetic progenitor model of tumourigenesis”

Potential transgenerational epigenetic effects?

Zebrafish



F0 Males

Untreated

F0 Females

1. Untreated
2. 5-AZA
3. MeHg
4. TCDD

n=6

Global DNA methylation
MeDIP-chip
RT-qPCR

F1

1. Untreated
2. 5-AZA
3. MeHg
4. TCDD

n=6 pools

Global DNA methylation
MeDIP-chip
RT-qPCR

F2

1. Untreated
2. 5-AZA
3. MeHg
4. TCDD

n=6 pools

Global DNA methylation
MeDIP-chip
RT-qPCR

24 tanks

4 treatments

6 tanks per treatment

Timeline and sampling

Start - September 9

Dietary exposure
6 weeks

F0 Sampling
October 26/2011

Liver: DNA/RNA
Whole fish: MeHg/TCDD

No exposure

F1 - November 3/2011

Embryos
3 DPH
pools n=10

No exposure

F2 - February 20/2012

Embryos
3 DPH
pools n=10

Impacts of TCDD and MeHg on DNA methylation in zebrafish (Danio rerio) across two generations.
Olsvik et al. Comp Biochem Physiol C Toxicol Pharmacol. 2014. doi: 10.1016/j.cbpc.2014.05.004.

Conclusion

- The epigenetic mechanisms have a significant role in regulating the responses of aquatic species to chemicals including carcinogens.

- Epigenetic "foot-printing" of organisms could identify classes of chemical contaminants to which they have been exposed throughout their lifetime. This information can be used to assess the impact of environmental conditions on organisms.

- Finally, It is recommended that epigenetic mechanisms, alongside genetic mechanisms, should eventually be considered in environmental toxicity safety assessments and in biomonitoring studies. This will assist in determining the mode of action of toxicants, no-observed-adverse-effect level (NOAEL) and identification of biomarkers of toxicity for early detection and risk assessment in toxicology.

Mirbahai and Chipman (2014) Epigenetic memory of environmental organisms: a reflection of life-time stressor exposures. **Mutation Research**: **Genet. Toxicol. Environ. Mutagen**. 764-765: 10-17

Acknowledgements

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