



Supplementary Tables S1, S2, and S3

Table S1. Examples of studies demonstrating progressive changes in DNAm leading to cancers, or field effects.

Progressive Changes		
Cancer Type	Progression	Authors
Lung cancer	Global genome DNA hypomethylation in tumor-associated fibroblast and non-small cell lung cancer.	[1]
Melanoma	From benign nevi to malignant primary lesions to metastasis (also describe inactivation of cell-adhesion, then activation of inflammatory and immune system impairments).	[2]
Gastric cancer	From gastritis (<i>H. pylori</i>) to metaplasia and gastric cancer.	[3;4]
Oral squamous cell carcinoma (OSCC)	Within patient's progression from normal biopsies adjacent to dysplasia, to carcinoma <i>in situ</i> (CIS) and OSCC.	[5]
Oral squamous cell carcinoma (OSCC)	From chronic inflammatory disease (chronic periodontitis) to pre-neoplasia.	[6]
Field effects*		
Bladder cancer	L1 hypomethylation in normal urothelium from bladder containing cancers and in bladder cancers.	[7]
Pancreatic cancer	Sat-II RNA expression in normal tissue, in non-cancerous tissues adjacent to pancreatic ductal adenocarcinoma.	[8]
Prostate cancer		[9]
Colon cancer	Normal tissue surrounding colon cancer.	[10]
Ovarian cancer	L1 hypomethylation and expression in normal-appearing fallopian tube epithelium, in serous tubal intraepithelial carcinomas, and in ovarian carcinomas.	[11]
Breast cancer	Gene specific methylation in tissues surrounding cancer.	[12]
		[13]

*Field effects support epigenetic disruption as an early event in carcinogenesis, or as marker of the presence of a cancer in the surrounding tissues.

Table S2. Histone post translational modifications (HPTM) induced by arsenic in short- and long-term experiments.

Model	Type	Treatment		Effects	Outcome	Authors
		Dose	Length			
CD-1 mice lungs and liver	In vivo	Dimethylarsinic acid (DMA), 200 ppm	Trans-placental gestation day 8-18	84 weeks of age. In the lung: ☑ H3K9me2, but no effect on H3K27me3	Increase incidences of total tumours, of lung adenocarcinomas, and of hepatocarcinomas in males.	[14]
Human blood lymphocytes	In vivo	Ranked in four groups from no signs to severe cases of arsenicosis	Chronic	☑ H3K36me3 from mild to severe cases. ☑ H3K9me2 from mild to severe cases. ☑ H4K20me2 in severe cases	Arsenicosis	[15]
	In vivo	Low vs high inorganic arsenic (monomethylarsonous acid) exposure (10–30 µg/l vs > 150 µg/l in drinking water)	Chronic	☑ H3K18ac ☑ H4K8ac No significant changes for H3K23ac and H4K12ac.		[16]

HaCaT Human keratinocytes	Cell line	Sodium arsenite (NaAsO ₂) 0, 1.25, 2.5, 5, or 10 μ M	24h	<input type="checkbox"/> in global genome H3K9me2, but <input checked="" type="checkbox"/> H3K9me2 in promoter of MPG, XRCC1, and PARP1, reducing their expression.	Arsenic reduce expression of base excision repair genes (MPG, XRCC1, and PARP1) aggravating DNA damage.	[17]
	Cell line	Sodium arsenite (NaAsO ₂) 0.0, 2.5, 5, 10, and 20 μ M	24h	<input type="checkbox"/> H3K18ac <input type="checkbox"/> nucleotide excision repair related genes (XPA, XPD, and XPF).	Increase in DNA damage.	[18]
L-02 Normal human liver cells	Cell line	Sodium arsenite (NaAsO ₂) 0, 5, 10, 20 μ M	24h	<input checked="" type="checkbox"/> KDM3A/JHDM2A <input type="checkbox"/> global H3K9me2 <input checked="" type="checkbox"/> H3K9me2 in promoters of base excision repair genes	Increase in DNA damage.	[19]
HepaRG human non-tumorigenic liver cells	Cell line	Sodium arsenite (NaAsO ₂) 1 μ M	14 days	<input checked="" type="checkbox"/> Global genome DNA hypomethylation. <input checked="" type="checkbox"/> Site specific DNA hypermethylation (e.g. CLDN14). <input type="checkbox"/> H3K36me3. <input checked="" type="checkbox"/> H4K20me3. <input checked="" type="checkbox"/> DNA damage.	Induction of carcinogenesis-related events, epithelial-to-mesenchymal transition based on gene expression, damage to DNA, inhibition of DNA repair genes. No effect on H3K4me3, H3K9me3, H3K27me3, H3K9ac, H3K27ac, H4K16ac	[20]
A/J mice lungs	In vivo	Sodium arsenite NaAsO ₂ , 100 μ l behind the tongue every other day for one week from 100 and 200 μ g/L solutions		<input checked="" type="checkbox"/> H3.1 mRNA. <input type="checkbox"/> SLBP mRNA and proteins. In nude mice, FH3.1poly(A)-expressing BEAS-2B cells were tumorigenic.	Confirmed <i>in vivo</i> effects of arsenic observed <i>in vitro</i> .	[21]
BEAS-2B human broncheal epithelial cell line	Cell line	Sodium arsenite NaAsO ₂ , 0.5 and 1 μ M	96h	<input checked="" type="checkbox"/> polyadenylation of H3.1 mRNA. <input checked="" type="checkbox"/> H3.1 protein. <input type="checkbox"/> H3.3 at promoters, enhancers, insulators. Effects of arsenic on polyadenylation counteracted by SLBP expression.	Transcriptional deregulation, cell cycle arrest, aneuploidy, chromosomal aberrations, colony formation in soft agar transformation.	[21]
	Cell line	20 μ M As ³	2-24h	No effect on H3K27me3. As ³⁺ induces EZH2* S21 phosphorylation in BEAS-2B through JNK and STAT3-dependent Akt activation. In As ³⁺ increase expression of miR21 which	This work reveal a signaling cascade induced by As ³⁺ within less than 24h that may prevent normal methylation of H3K27. In response to As ³⁺ treatment, JNK	[22]

				downregulate Spry2 (a negative regulator of Akt). As ³⁺ induce cytosolic location of phosphorylated EZH2-S21.	phosphorylates STAT3 leading to increased expression of miR-21, which, in turn, downregulates the expression of Spry2 (a negative regulator of Akt signaling). The activated Akt phosphorylates EZH2 at S21 that mostly remain cytosolic. A reduction in EZH2 in the nuclei may reduce abundance of H3K27me3 and activate oncogenes.	
A549 Human lung carcinoma cells	Cell line	1-5 μM As ³	24h	\downarrow H3K4me1. \uparrow H3K4me2, me3.		[23]
		0.1, 0.5, 1 μM	7d	\uparrow H3K4me3 abundance.		
	Cell line	Sodium arsenite (NaAsO ₂) at 1,3,10 μM (As ³). Monomethylarsine oxide at 0.3,1,3 μM (MMA ³)	24h 7d	\downarrow H4K16ac at 3 μM MMA ³ and 10 μM As ³ . \downarrow H4K16ac at 0.3 μM MMA ³ and 1 μM As ³	\downarrow KAT8 (MYST1/hMOF) can sensitize cells to arsenic toxicity	[24]
UROtsa Human urothelial cells (SV40 transformed, non tumorigenic)	Cell line	50 nM MMA ³⁺	16 weeks	Specific study of H3K18ac over the genome.	Alteration of histone acetylation patterns in a time- and malignant stage-dependent aberrant gene-expression pattern.	[25]
	Cell line	50 nM MMA ³⁺	Time-series 4, 8, 10, 12, and 14weeks	\uparrow H3 ac and \downarrow H4 acetylation at the time of malignant transformation. Investigated series of H3 and H4 lysine acetylation and H3 methylation.	Malignant cell transformation; colony formation in soft agar and nude mice xenograft assay	[16]
HEK293T human embryo kidney or HELA1	Cell line	0.2-0.8 μM arsenic trioxide As ₂ O ₃	24h, 48h, 72h	\downarrow H4K16ac. \downarrow HAT act MYST1 via As-Zn finger domain direct interaction		[26]
NHEK cells, primary culture of normal human epithelial. Keratinocytes.	Primary	0.5 μM As ³	24h up to 10 weeks	\uparrow H4K16ac from Day 1 to 48. DNA hyper and hypomethylation of SIRT1** and pri-miR-34a, from week 5 to 10.	Cumulative disruptions to epigenetic regulation of miR-34a expression, SIRT1, polycomb repressive group complex, and	[27]

Downregulation of SIRT1 p53 functional activi-
and miR-34a over the ties.
first 3 weeks.

*EZH2 is the enzymatic subunit of the PRC2 complex responsible for the trimethylation H3K27me3. EZH2 S21 phosphorylation facilitates the dissociation of the PRC2 complex which may reduce the methyltransferase activity of EZH2 toward H3K27. **SIRT1 is recruited to methylation sites as part of polycomb repressive (PRG) complexes 2 and 4, where it associates with and regulates EZH2, EED, SUZ12 and DNMT1. SIRT1 coordinates chromatin remodeling during oxidative DNA damage by deacetylating H4 at Lys 16 (H4K16) and maintains cytosine methylation by recruiting and activating DNMT1. miR-34a is a regulatory component of p53 mediated signaling. miR-34a whose expression is induced by p53-mediated transcription activation) regulates expression of SIRT1 by base pairing with its 3'UTR. p53/SIRT1/miR-34a form a coherent feed-forward loop (the p53/SIRT1/miR-34a axis).

Table S3. Arsenical exposures revealing altered DNA methylation mechanisms.

Substance	Treatment duration	Cell/tissue type	Endpoint	Authors
Arsenic trioxide (As ₂ O ₃)	48 h 2 to 10 µM	HepG2	Decrease radiometric DNMT activity on artificial substrate & DNMT mRNA	[28]
Sodium arsenite (NaAsO ₂)	24 h 25 µM	HaCaT	Decrease SAM	[29]
Sodium arsenite (NaAsO ₂)	72 h 0.5, 1.5, and 5 µM	HaCaT	Decrease in DNMT1 and 3a mRNA	[29]
Sodium arsenite (NaAsO ₂)	8 weeks	BEAS-2B	DNMT1, 3a, 3b, mRNA & protein	[30]
Sodium arsenite (NaAsO ₂)	8 weeks 0.5 µM	BEAS-2B	Increases in TET enzyme expression. Redistribution and increase in global 5hmC. EMT based on mRNA.	[31]
Sodium arsenite (NaAsO ₂)	24 h 1.0 µM	HaCaT	DNA methylation dependent expression of let-7 miR family member	[32]
Sodium arsenite (NaAsO ₂)	16 weeks 2.5 µM	p53 ^{low} HBEC	EMT, DNA methylation suppression of miR200	[33] [34]
As human blood contaminant	Chronic	Blood samples	Review of LINE-1, Alu, global genome demethylation	[35]

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