

Peter Jones

Structure 1

5'-CG^{5m}CGAATT^{5m}CGCG-3' NDB ID: BDLB72



Structure of B-DNA







Eukaryotic Methylation



Eukaryotic Methylation on Inactive X- Chromosome





Nature <u>429</u> 457- 463 (2004)

Eukaryotic Methylation



Eukaryotic Methylation in Cancer



Chromosome in Normal Cell



Chromosome in Cancer Cell

Epigenetic Therapy









Nature <u>429</u> 457- 463 (2004)



Treatment of T24 bladder cancer cells with **Decitabine** causes a reduction of DNMT protein levels and activation of *p16*







after course 3



Abnl. Metaph.

20/20

Genes Induced > 4 Fold by 5-Aza-CdR

Gene Class	LD419 (Fibroblast)	T24 (Tumor)	Overlap
Interferon induced	1	10	
Cytokine	2	7	SAA1
Membrane proteins	2	6	
MAGEs	1	5	MAGE4
Growth factors	1	4	
Cytokeratins	4	1	KRT17
Histones	3	3	H2AFL, H2BFH
Transcription factors	1	3	STAT1
Protease inhibitors	0	3	
Proteases	3	2	
Complement component	1	3	C3
Imprinted gene	1	1	H19
Apoptosis	0	2	
Miscellaneous	13	12	

Total induced genes 34/6,600 (0.5%) 61/6,600 (0.9%)

Genes inducible by interferon

<u>10/34 (29%)</u>

33/61 (54%)

Problems with Epigenetic Therapy

- Remethylation
- Labile Drugs
- Selectivity

Re-silencing of *p16* Gene after 5-Aza-CdR Treatment





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Chemical Structures





Covalent binding of DNMTs to zebularine-substitued DNA



David Hornby

Kinetics of *p16* gene expression after zebularine treatment in T24 cells

Days After Treatment (500 µM



Continuous Treatment of T24 cells with



Reactivation of *p16* in EJ6 Tumor Cells Subcutaneously Implanted in Mice



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INCORPORATION OF ¹⁴C-ZEBULARINE IN DNA AND ACTIVITY OF URIDINE/CYTIDINE KINASE



Depletion of DNMT proteins after continuous treatment with zebularine



<u>Preferential response of</u> <u>cancer cells to DNA methylation inhibitors</u>

	Fibroblasts		Cancer Cells	
	↑	\downarrow	↑	\downarrow
5-Aza-CdR (≥4 fold)	34/6,600	11/6,600	61/6,600	2/6,600
Zebularine (≥2 fold)	1/13,000	1/13,000	12/13,000	6/13,000

Microarray Analysis of Fibroblasts and Cancer Cells After Continuous Zebularine Treatment



Gene		Fold Change		
	MAGEA1 MAGEB2 GAGE3 GAGE6 GAGE7 GAGE7B XAGE1 SPANXA1	6.0 3.2 5.7 8.0 9.6 12.4 21.2 258.3		

Methylation and Expression of XAGE1 after Continuous Zebularine Treatment





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