PRMT1 PROJECT

Methylation of arginine residues of the proteins is one of the least charachterized post-translational modification[1]. An early hypothesis in the field raised by Albert Szent-Györgyi suggested that dimethylation of arginine and lysine residues protects proteins against reactive moieties [2].
We have found that arginine methylation plays a key role in the regulation of gene expression [3]. Our studies indicate that in the retinoid driven differentiation of a myeloid cell line methylation of arginine residues on histone H4 is markedly increasing retinoid responsiveness [3]. During our further studies we have identified arginine methzlation foci in the genome [4]. ES cells lines were isolated from E3.5 blastocysts and were maintained on 0.1% gelatinized tissue culture plates in ES cell medium.

The key arginine methyltransferase of mammalian cells is PRMT1 (**P**rotein A**r**ginine **M**ethyl**t**ransferase1). We plan to study the role of arginine methylation in differentiation by using an embryonic stem (ES) cell line that was generated after mutation of PRMT1 by using insertional mutagenesis [5]. PRMT1 mutant mice (C57BlJ6) were crossed for 3 generations into 129sv mice. F3 mice heterozygous were interbred, and blastocysts were collected at E3.5 and cultured.

PRMT1 null ES cells divide normaly and although present markers of ES cells, they are unable to differentiate. Mutant cell lines are morphologically similar to other ES cell lines and grow with doubling times similar to that of wild-type ES cells. In our experimental approach we plan to introduce the human PRMT1 enzime and its catalytic mutant into these ES cells by using a tet-operon controlled lentiviral system. By reintroducing PRMT1 we plan to bypass the block in the differentiation in a well controlled manner. The level of arginine methylation will be monitored by using a HIV1-Luc reporter vector [6].

Differentiation of ES cells by nuclear receptor ligands can be driven in a variety of directions from neural trough bone and hematopoetic till adipose direction. By understanding the role of arginine methylation in differentiation of ES cells we hope to find new regulatory foci of nuclear receptor driven differentiation pathways.

A. Background

1. Methylation on arginine residues by PRMT1 (protein arginine methyltranferase 1) is modulating the activity of nuclear receptors and protects proteins against reactive groups.
2. Retinoid signaling is modulated by arginine methyltransferases.
3. Retinoids are involved at various steps of embryonic development. Retinoids and other nuclear receptor ligands are easy to use in differentiation cocktails
4. PRMT1 KO animals die at E 6.5, but “embryonic stem” cells derived from these embryos are dividing and viable [5].
5. Basic differentiation of wild type ES cells by LIF removal is inducing CYP26 production and a natural active retinoid - the 4-oxo retinoid - synthesis.

B. Available reagents and methods

1. Wild type and PRMT1 KO mouse ES cells
2. hPRMT1-HA tagged expression vector and its catalytic mutant
3. tet-KRAB conditional lentiviral system with GFP marker [7] and anti hPRMT1 siRNA insert [8].
4. Specific PRMT1 inhibitor AMI1.
5. MS/MS method for detection of 4-oxo retinoids.

C. Questions to be addressed

1. Is ES cell differentiation linked directly to retinoid signaling through the cofactor of the retinoid receptor PRMT1?
2. What is causing the block in differentiation of PRMT1-/- ES cells? Is there a block in retinoid production or retinoid receptor activity? Is there endogenous retinoid production in the lack of PRMT1?
3. What is the role of PRMT1 in differentiation of ES cells after the stage corresponding to E6.5?
4. How can we modulate ES cell differentiation if we bypass the first block in differentiation (corresponding E6.5) using tet-KRAB controlled expression of hPRMT1?
5. Can we dissect the enzymatic role of PRMT1 from the non-enzymatic transcriptional activator role?
6. How can we exploit the specific PRMT1 inhibitor (AMI1) in ES cell differentiation?

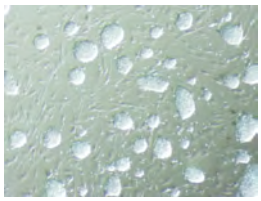
A.  B.  C. 

Figure 1. A. ES cells on mitomicyn treated MEF in non differentiating medium. B. ES cells on gelatinized plate after 72 hours of LIF withdrawal. C. Embrioid body formed in hanging drop culture.

Funding: OTKA 68254 Young Investigator grant

References:

1. Kouzarides, T., *Histone methylation in transcriptional control*. Curr Opin Genet Dev, 2002. **12**(2): p. 198-209.
2. McLaughlin, J.A., R. Pethig, and A. Szent-Gyorgyi, *Spectroscopic studies of the protein-methylglyoxal adduct*. Proc Natl Acad Sci U S A, 1980. **77**(2): p. 949-51.
3. Balint, B.L., et al., *Arginine methylation provides epigenetic transcription memory for retinoid-induced differentiation in myeloid cells*. Mol Cell Biol, 2005. **25**(13): p. 5648-63.
4. Balint, B.L., P. Gabor, and L. Nagy, *Genome-wide localization of histone 4 arginine 3 methylation in a differentiation primed myeloid leukemia cell line*. Immunobiology, 2005. **210**(2-4): p. 141-52.
5. Pawlak, M.R., et al., *Arginine N-methyltransferase 1 is required for early postimplantation mouse development, but cells deficient in the enzyme are viable*. Mol Cell Biol, 2000. **20**(13): p. 4859-69.
6. Kwak, Y.T., et al., *Methylation of SPT5 regulates its interaction with RNA polymerase II and transcriptional elongation properties*. Mol Cell, 2003. **11**(4): p. 1055-66.
7. Szulc, J., et al., *A versatile tool for conditional gene expression and knockdown*. Nat Methods, 2006. **3**(2): p. 109-16.
8. Rezai-Zadeh, N., et al., *Targeted recruitment of a histone H4-specific methyltransferase by the transcription factor YY1*. Genes Dev, 2003. **17**(8): p. 1019-29.