METHYLATION OF PEG10 ALLELES IN EWING SARCOMA

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Ewing Sarcoma (ES) is the second most common bone cancer (1), and is described as a small, round, blue cell tumor. ES is usually characterized by a translocation and thus creation of the EWS-FLI1 fusion protein (2). However, there are other chromosomal aberrations that have been implicated in ES, such as trisomy 8 (3). Our lab has shown that a subset of those with trisomy 8 have an upregulation of PEG10. PEG10 is an imprinted paternally expressed gene. The paternal allele is expressed while the maternal allele promoter is methylated and thus the maternal allele is silent and unexpressed. PEG10 is also expressed in and essential for placental development. The mechanism by which PEG10 is upregulated in the trisomy 8 subset is unknown. We hypothesized two possibilities resulting in upregulation of PEG10: 1) The maternal allele promoter remains methylated, and the paternal allele is upregulated alone, 2) the maternal allele promoter becomes un-methylated and thus both paternal and maternal alleles are expressed resulting in the upregulation pattern. In order to quantify methylation of PEG10 alleles, bisulfite sequencing will be used. Bisulfite sequencing is a common technique used to evaluate methylation of CpG islands (4). In theory, sequence reads should show us which of the two hypothesis is representative of the PEG10 upregulation pattern. A ratio of 1:1 of un-methylated reads to methylated reads of the promoter regions should tell us that hypothesis 1 may be the mechanism involved, whereas all un-methylated reads may point towards the mechanism involved in hypothesis 2.

Bisulfite sequencing works by converting all un-methylated Cs to Ts leaving the methylated Cs unchanged. Thus when obtained sequences are compared to the original sequence, areas of methylation can be determined. We chose 3 islands in the promoter region of PEG10 to bisulfite convert, amplify via PCR, and sanger sequence (Fig. 1)(5).

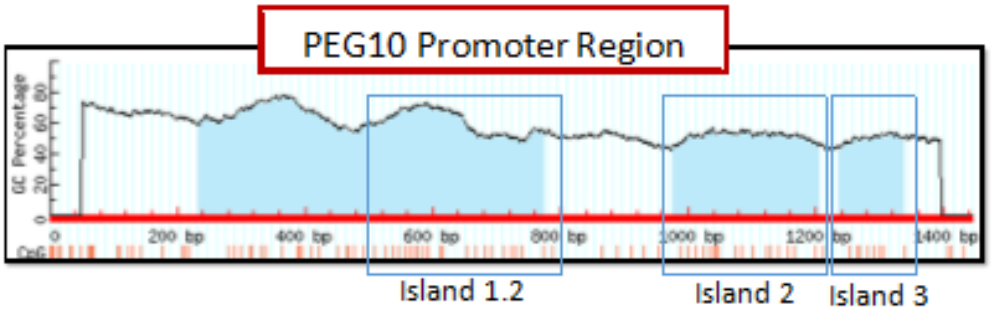

Figure 1: Promoter region of PEG10 with island locations amplified via PCR.

Sequencing reads ratios were analyzed for cell lines with and without expression of PEG10 (A673 + expression, CHLA9 – expression, TC252 + expression, Utes1 + expression, HepG2 + expression positive control, Placenta PEG10 expression undetermined). Island 3 seems to be completely methylated across both alleles and therefore may not be involved in imprinting patterns (Fig. 2A). Island 2 appears to follow the canonical imprinting pattern (Fig. 2B). Island 1.2, however does not seem to have a clear pattern (Fig. 2C) and this may be the sight of control of differing PEG10 expression in our cell lines.

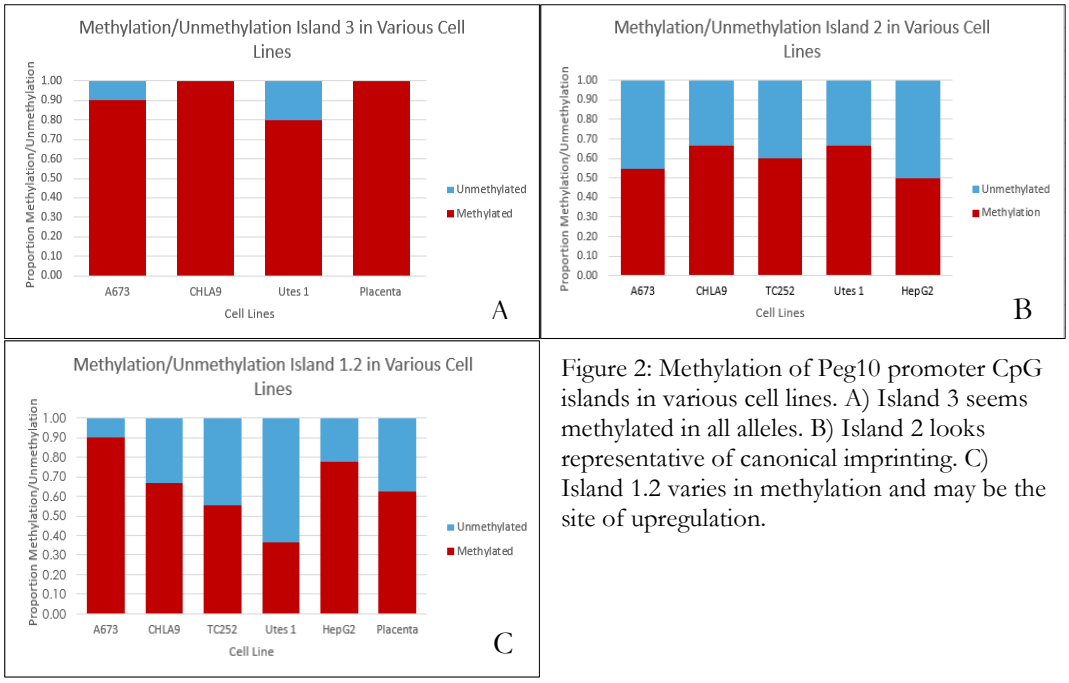

Figure 2: Methylation of Peg10 promoter CpG islands in various cell lines. A) Island 3 seems methylated in all alleles. B) Island 2 looks representative of canonical imprinting. C) Island 1.2 varies in methylation and may be the site of upregulation.

We were curious if the levels of PEG10 mRNA matched the methylation patterns predicted by Island 1.2. Using qRT-PCR, PEG10 mRNA levels were quantified (Figure 3). HepG2 is a hepatocellular carcinoma and overexpresses PEG10 (6), and we used this as our positive control.

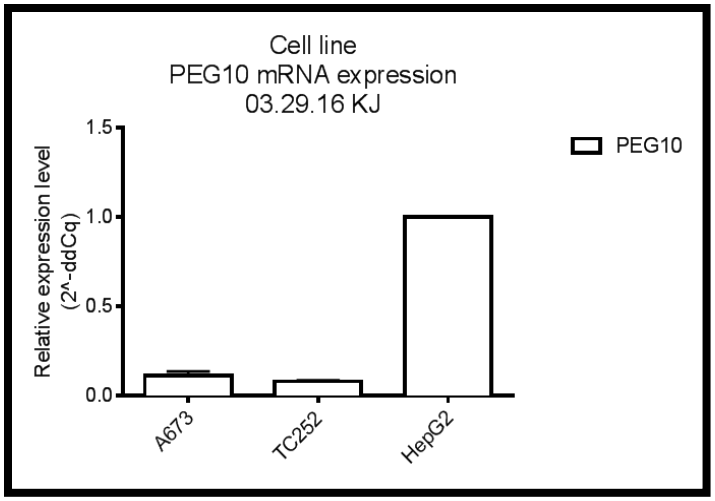

Figure 3: PEG10 mRNA expression levels determined via qRT-PCR. Normalized to HepG2.

PEG10 mRNA expression and protein expression levels in our cell lines are consistent yet they contradict what we would expect based on our methylation data. This could be explained by PEG10 being upregulated by some other mechanism. Alternatively, the methylation patterns observed could be methylation lost between cell divisions and not necessarily reflect the separate alleles. We must determine which of these scenarios are seen in the methylation results. If the methylation results do reflect two alleles, then another mechanism of upregulation must be at play and needs to be determined. Other research suggests that there are other

transcription factors that upregulate PEG10 in hepatocellular carcinoma (7), and these transcription factors can be upregulated in Ewing Sarcoma (8).

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