Epigenetics Tells the Mole Story: DNA methylation identifies complete moles destined to develop into gestational trophoblastic neoplasia

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Objectives: There is no method to predict which patients with complete hydatidiform mole (CHM) will develop gestational trophoblastic neoplasia (GTN). DNA methylation is a key regulatory mechanism in trophoblast development. This study sought to determine if DNA methylation distinguishes CHM destined to develop GTN. Array based bisulfite sequencing provides the ability to study whole genome methylation and enables discovery of epigenetic biomarkers and therapeutic targets.

Methods: Genomic DNA was extracted from 22 CHM specimens collected at the time of uterine evacuation. Bisulfite modification and methylation patterns were investigated using the Illumina HumanMethylation450 array, and validated by bisulfite sequencing for selected probes. Two-way hierarchical clustering was performed and the degree of methylation at sites throughout the genome were compared between samples. Quantitative comparison between sample clusters was used to identify biologically relevant differentially methylated regions (DMRs). Logistic regression was then used to develop models based on all 181 significantly differently methylated CpG sites. The Delta mean was defined as the difference between GTN and CHM averaged beta values. Overfitting of the models was assessed in leave-one-out cross-validation (LOOCV).

Results: Among the 22 patients, 13 had hCG normalization without treatment, and 9 developed GTN that required methotrexate. One methotrexate resistant case required second line therapy with pulse-ActD. Distinct methylation signatures were observed between benign CHM and CHM that progressed to GTN after two-way hierarchical clustering of DMRs (Figure 1). 182 distinct genomic regions exhibited significantly different levels of methylation. 133 DMRs were hypomethylated and 47 were hypermethylated in CHM cases that progressed to GTN. DMRs were more likely to occur in coding regions followed by intergenic regions. Notable DMRs between CHM progressing to GTN compared to benign CHMs include multiple PI3K/AKT pathway members: RPTOR (2.3 Fold increase, p=0.022), PIK3C2G (1.7 Fold decrease, p=0.038) and PEBP4 (1.8-fold increase, p=0.003). TGF-β receptor SMAD3 (2.0 Fold decrease, p=0.05) and NOTCH receptor DLL1 (2.5 Fold increase, p=0.008) were also notably altered. LOOCV validated 6 DMRs located in the LRIT2, ELAVL2, MORN2, C6orf10, HDAC4 and intergenic region at CpG cg15935392 that robustly separate progressing CHM cases and benign CHM

Conclusions: This is the first study to identify epigenetic markers that predict progression to GTN in CHM tissue collected at initial uterine evacuation. DMRs between these populations of CHM not only predict new biomarkers and therapeutic targets for post-molar GTN, but also suggest DNA methylation is fundamental to tumorigenesis in GTD.

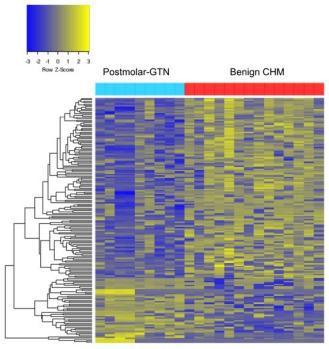

Figure 1 Two-way hierarchical clustering of genomic methylation patterns reveal distinct signatures that predict disease progression