Epigenetic Changes Influence Genome-Wide Binding Of Myod in Rhabdomyosarcoma

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Rhabdomyosarcoma (RMS) is a pediatric cancer of skeletal muscle that is thought to occur because of a failure of myoblasts to fully differentiate. The DNA binding protein MyoD drives the differentiation of myoblasts into terminally differentiated myotubes; however, it is expressed normally in RMS cells. It is therefore unclear why RMS cells remain immature. In a study published in Molecular and Cellular Biology, graduate student Kyle L. MacQuarrie (Human Biology Division) and Dr. Stephen J. Tapscott (Human Biology and Clinical Research Divisions) use a genome-wide approach to identify differences in MyoD binding between RMS and normal muscle and epigenetic differences that may influence myotube differentiation.

MyoD is a basic helix-loop-helix (bHLH) transcription factor, which heterodimerizes with E-protein to transactivate gene expression and promote muscle cell differentiation. Previous studies in mice demonstrated that MyoD binding sites are ubiquitous across the genome in both myogenic stem cells and differentiated myotubes, often near potential binding sites for other myogenic factors such as Runx and Sp1. Furthermore, gene expression during differentiation is positively correlated with the level of MyoD binding near a given gene. These observations led the team to ask whether or not MyoD binds DNA differently in RMS than in normal cells.

To answer this question, the team performed ChIP-seq analysis for MyoD in primary human myoblasts and myotubes, as well as RD cells, a model for RMS. The team found that 30-60,000 sites were occupied by MyoD across the genome of both human myoblasts and myotubes. 50-90% of these sites are occupied by MyoD in both cell types, and gene expression was positively correlated with the level of MyoD binding. Surprisingly, most of these MyoD binding sites were also occupied in RD cells. However, the team did find two types of differences; regional differences, in which areas >100 kb had consistently higher or lower MyoD binding profiles, and local differences, in which an individual binding site, but not neighboring sites, displayed differential binding profiles. ENCODE project data showed that myoblast genomic regions which bind lower levels of MyoD than RD cells are also less susceptible to DNase I at these sites. This observation suggested that chromatin accessibility may influence MyoD binding in these regions. Supporting this hypothesis, team found that RD cell Pvull cleavage sites in regions with reduced MyoD binding were less
susceptible to digestion relative to the same region in normal myoblasts with a stronger MyoD binding signature.

A motif analysis of sequences adjacent to MyoD sites differentially bound in myotubes and RD cells found enrichment of RUNX1 and AP-1 binding sites, which are sufficient to drive differentiation of RD cells, NFIC sites, which bind bHLH co-factors in myogenic cells, and MEF2 sites which bind MyoD at muscle-specific promoters. When the authors overexpressed MEF, they observed increased MyoD recruitment to MEF2C sites. RT-PCR and expression analysis confirmed that RD cells expressed fewer transcripts of these myogenic cofactors than primary myotubes, suggesting that underexpression of these transcripts may contribute to the differentiation block in RD cells. The Tapscott lab found that overexpression of the AP-1 family member JDP2, or NFIC led to cell cycle withdrawal and increased expression of the MEF2C transcription factor, while overexpression of MEF2C alone led to increased differentiation of RD cells into myotubes.

Taken together, these data indicate that epigenetic changes in RMS cells result in decreased expression of some MyoD co-factors and their regulated genes. Overexpressing these co-factors rescued muscle differentiation in RD cells, supporting the hypothesis that RMS is a failure of myoblasts to reach the threshold concentration of myogenic factors necessary to induce differentiation. Furthermore, this work suggests that if this threshold is crossed, normal myogenic differentiation may proceed in RMS cells. “The fact that multiple factors can be utilized to cause cell cycle withdrawal and myogenic differentiation in the tumors - due to the tightly regulated nature of the control of myogenic differentiation - is encouraging for the ability to find one or more drug-able targets,” said Kyle L. MacQuarrie.

Position weight matrix (PWM) of the MEF2C binding motif - a higher PWM indicates a potential MEF2C binding site that looks more 'perfect' than a site with a lower PWM score. Genes that are expressed more strongly and associated with more MyoD binding in myotubes compared to RD cells (pink) have a stronger association with canonical MEF2C binding motifs (MEF2C PWM) than those genes that have comparable expression and MyoD binding between myotubes and RD cells (blue).

Image courtesy Kyle L. Macquarrie