Professional Development Grant Final Report

Arkansas Tech University

Application of Ultra-High-Throughput RNA Sequencing to Identify the Role of TACC2s in Rhabdomyosarcoma

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B. Research Problem Investigated.

Rhabdomyosarcoma (RMS) accounts for 4-8% of all childhood cancers. RMS treatment strategies have been hampered by a lack of knowledge of the steps leading to the formation of the tumor. We have previously shown that failure to turn off the TACC2s isoform in a muscle cell precursor (myoblasts) prevents normal muscle differentiation, at least in part by preventing the upregulation of MyoD, a key regulator of muscle differentiation. Thus, dysregulation of TACC2s directly results in conditions predicted for rhabdomyoblast stem cells that progress to malignant RMS. However the mechanism by which TACC2s inhibits normal muscle development is currently unknown. The goal of this proposal was to use a new ultra-high-throughput technique called RNA sequencing to identify genes regulated by TACC2s and thus new control pathways that misregulate the muscle developmental program resulting in RMS.

C. Research procedure.

The muscle cell developmental program was triggered in confluent cell culture by withdrawal of serum from myoblast cells overexpressing TACC2s (pcDTACC2) and the control myoblasts. We chose to harvest total RNA 2 days afterward as this is the point at which MyoD induction should normally occur. RNA was prepared for RNA sequencing using a Qiagen RNAEasy kit, and quality control of the samples was carried out as required for subsequent RNA sequencing.

RNA sequencing rapidly sequences short stretches of every RNA in the cell and thus permits a relatively complete, unbiased gene expression survey. The original proposal was to send samples to Otogenetics Corporation to perform an 8 million sequence-read "pilot study". However, while the tissue culture and RNA preparation was being carried out, Otogenetics offered increased coverage, at a lower cost than originally budgeted. Thus, more in-depth coverage of 20 million sequence reads was able to be performed to generate expression profiles of the 20,000 genes that can be expressed in a given cell type. Otogenetics also performed basic bioinformatic analysis to determine the quantitative differences in gene expression, and alternative splicing between the normal and TACC2s overexpressing myoblasts.

D. Summary of Findings.

Initial analysis by Otogenetics identified 1090 genes that demonstrated statistically different expression between the normal, differentiating myoblasts and the differentiation-arrested TACC2s myoblast cell lines. These genes were then sorted and grouped using the DAVID Bioinformatics Resources (http://david.abcc.ncifcrf.gov/) based on their biological function and involvement in developmental pathways. Of particular interest were changes in transcription factors such as HOPX, HEYL and MYF6, and chromatin remodeling factors such as SMARCA4 and MeCP2, which have a potential to directly affect the expression of myogenic regulators such as MyoD. Further in-depth bioinformatics analysis was facilitated by a free trial of the Ingenuity Systems Pathway Analysis system (http://www.ingenuity.com). This latter, more global analysis further identified changes in the expression of genes in the Notch, Wnt and STAT signaling pathways, confirming relatively recent data published on these pathways and their significance in myogenesis. In addition, IPA Upstream Regulator Analysis has predicted potential "nodal" proteins, whose expression did not change, but which may represent the proteins through which the TACC2s protein directly interacts to perturb muscle cell differentiation. Intriguingly, Ingenuity also identified aberrations in pathways that lead to cardiac hypertrophy, suggesting a role for TACC2s in heart disease that also merits further experimental investigations.

E. Conclusions

Clearly, this Professional Development Grant has afforded a significant advance in my research into the function of TACC2 and the mechanism by which TACC2s could disrupt normal muscle cell development, and thus contribute to the initiation and progression of the pediatric cancer rhabdomyosarcoma. In Fall 2012, this research captured the interest of two students in the BS Biology- Biomedical option who, through an investment from the ATU Undergraduate Research Fund, have already confirmed, using wet lab techniques, aberrations in the expression of several of the key genes from the bioinformatic analysis. Preparation of an abstract for oral and poster presentation at the 2013 Arkansas Academy of Science is currently underway.