Anisley Valenciaga
PhD Candidate

“Cell cycle regulators and transcriptional targeting in Medullary Thyroid Cancer”

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BRT 134
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VITA

1988 .................................. Born – Havana, Cuba

2009 .................................. A.A., Miami Dade College

2012 .................................. B.S., Florida International University

2012-Present .......................... Medical Scientist Training Program, The Ohio State University

2016-Present .......................... F31 Fellow

COMMITTEE MEMBERS

Matthew D. Ringel, MD

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ABSTRACT

Medullary thyroid cancer (MTC) is a currently incurable disease. FDA approved therapies that target RET, a commonly mutated tyrosine kinase in MTC, do not offer complete responses and patients acquire resistance over time. The CDK/RB cell cycle pathway shows potential as a different target for MTC given that LOH at the p18 and E2F2 loci has been found in a small cohort of human MTCs, and loss of rb and negative regulators of this pathway in mice results in MTC occurrence. In addition to the “classical” CDK pathway, targeting transcription regulation using CDK9 and CDK7 inhibitors have been shown to reduce tumor burden in pre-clinical studies of difficult-to-treat tumors, but not MTC. The objective of this study was to determine if cell cycle pathways represent predictors of poor clinical outcome and/or are therapeutic targets for MTC, including treatment-resistant disease.

We tested whether reduced expression of RB and/or overexpression of hyperphosphorylated RB (pRB) as biomarkers of CDK activation predict MTC aggressive behavior in 56 MTC human cases. We found that on univariate analysis, reduced RB (<75% tumor cell staining) trended with lower MTC-specific survival for primary tumor and metastatic nodes (primary tumor HR 3.54 (95%CI: 0.81, 15.47), p=0.08 and lymph node HR 4.35 (95%CI: 0.87, 21.83), p=0.05). For primary tumors, multivariable analysis showed that low nuclear RB expression independently associated with worse disease-specific (p=0.01) and overall (p=0.02) survival. pRB levels were not associated with survival for either primary tumor or lymph node metastases. Thus, activation of this pathway appears to predict aggressive MTC behavior.
We then explored targeting of the CDK/RB pathway \textit{in vitro} using two MTC cell lines TT and MZ-CRC-1 with different RET driver mutations. CDK4/6 inhibition resulted in cell growth arrest but did not affect cell metabolic activity as a measure of cell viability or induce apoptosis. By contrast, targeting cyclin dependent kinases 1, 2, 5, and 9 using Dinaciclib was much more effective. Both cell lines were sensitive to Dinaciclib (IC50 at 72h: 0.0046\textmu M for TT and 0.0139\textmu M for MZ-CRC-1) and TT and MZ-CRC-1 cells with acquired resistance to Vandetanib retained sensitivity to Dinaciclib. With Dinaciclib treatment at the IC50 concentrations, CDK1/2/5 protein levels were maintained but unexpectedly, CDK9 protein and mRNA levels markedly decreased and CDK9 activity was reduced. RNA sequencing showed a global reduction of genetranscription in both cell lines after Dinaciclib treatment, particularly genes with RNA polymerase II-dependent transcription consistent with loss of CDK9 function. To further analyze this mechanism, the MTC cells were treated with THZ1, a CDK7 inhibitor. The cells were sensitive to this compound (IC50 at 72h: 0.02668\textmu M for TT and 0.08797\textmu M for MZ-CRC-1) and treatment resulted in loss of CDK9 expression. The specific reduction in CDK9 protein seen with Dinaciclib treatment was not due to changes in protein stability or proteosomal degradation, indicating a transcriptional regulatory mechanism. At the same time, \textit{in silico} findings predict a super-enhancer regulating CDK9.

We also describe further analysis of Dinaciclib and THZ1 treated cells, which revealed that these treatments also decreased RET protein and mRNA expression, similar to CDK9. RET is also predicted to have a super-enhancer motif \textit{in silico}, making this type of regulation a possible explanation for the specific effect of these treatments on RET as well. These treatments enhanced Ret activation in the surviving cells, consistent with oncogene addiction. This activation showed potential for combination therapies. Exploring this possibility, we treated MTC cells with
Dinaciclib and Vandetanib, and found synergistic effects on cell metabolic activity in addition to reduction of RET activation with Vandetanib treatment after Dinaciclib exposure.

In summary, we showed that reduced RB expression is associated with decreased patient survival in univariate and multivariable analysis, independent from patient age at surgery or cancer stage. Future studies involving larger MTC patient populations are needed to determine if lower RB expression levels may serve as a biomarker for aggressive disease in patients with MTC. We also determined that Dinaciclib and the CDK7 inhibitor THZ1 are active against naïve and Vandetanib-resistant MTC cells in vitro, alone and in combination with Vandetanib. Inhibiting CDK9 or CDK7 affected CDK9 and RET transcription and protein levels, indicating a potential role for transcriptional targeting in MTC treatment and suggesting the possibility of a super-enhancer mechanism regulating CDK9 and RET.
RECENT ABSTRACTS AND PRESENTATION

Valenciaga, A., Saji, M., Zhang, X., Yu, L., Bumrah, C., Ringel, M.D., Cyclin dependent kinases as targets in Medullary Thyroid Cancer. Poster presentation: ENDO Annual Meeting: Tumor Biology; 2018 March 17-20; Chicago, IL.
AWARDS AND HONORS

ENDO 2018 Early Career Forum travel award

FUTURE PLANS

I will be returning to medical school after finishing my PhD at OSU. After completing my MD training, I hope to join an internal medicine residency program and work as a physician-scientist in the future.
Biomedical Sciences Graduate Program
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