DISSERTATION ABSTRACT (cont'd)

Mounting clinical evidence has begun to expose limitations to HDACI therapy in thyroid cancer and other malignancies including acute myeloid leukemia, gliomas, and lymphoproliferative diseases. Despite their pre-clinical promise, HDACI-mediated resistance is a common phenomenon in a variety of cancers. Investigations into mechanisms of acquired resistance to HDACI therapy have highlighted the role of Nuclear factor kappa B (NFκB) signaling. This pathway has been shown to be activated in response to oxidative/genotoxic stress, and presumably to DNA damaging chemotherapies, such as HDACIs, contributing to a pro-tumorigenic microenvironment and intrinsic cancer resistance. Given these findings, we aimed to characterize the effect of HDACI therapy (VPA, SAHA and AB3) on NFκB activity in thyroid cancer. We demonstrate that each of these HDACIs activate functional NFκB activity, and that concomitant treatment with the NFκB inhibitor bortezomib diminishes HDACI-induced NFκB activity. Furthermore, we demonstrate that NFκB suppression by bortezomib and by means of specific inhibitors of other pathway intermediaries synergistically interact with VPA, SAHA and AB3 in follicular thyroid cancer to potentiate cell growth inhibition. These studies reveal that NFκB inhibition may serve as a useful adjunct to HDACI therapy in thyroid cancer.

Collectively this thesis work identifies Notch3 as a tumor suppressor in thyroid cancer, and moreover, as a novel prognostic and therapeutic target of activation in these malignancies. We demonstrate that Notch3 activation can be achieved by treatment with both approved and exploratory HDACIs, and that this approach may be an effective therapeutic option for treatment of these malignancies. Furthermore, we demonstrate that intrinsic resistance to HDACI therapy occurs through the activation of the NFκB pathway in thyroid cancer. This understanding provides us the therapeutic rationale for concomitant NFκB silencing in order to potentiate HDACI-induced lethality.

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CMP Cellular and Molecular Pathology

Yash Somnay

Program of the Dissertation Defense Seminar
for the Degree of Doctor of Philosophy
in Cellular and Molecular Pathology

"Targeted Activation of Notch3 Signaling in the Treatment of Advanced Thyroid Cancer"

CLINICAL SCIENCES CENTER

Thursday, June 11th, 2015

3:00pm Room 5001A WIMR

Research conducted in the lab of
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DISSERTATION ABSTRACT

Notch signaling is defined by an evolutionarily ancient cell interaction that controls developmental fate. Mammals possess four Notch isoforms (Notch1-4), which are expressed at the cell surface. Signaling is initiated when the extracellular portion of the Notch transmembrane protein binds with one of its associated family of ligands (Delta-1, -3, -4, Serrate, or Jagged-1, -2), causing a series of intra-cytoplasmic and -nuclear events that lead to the transcription of Notch target proteins. Downstream targets of Notch signaling are implicated in differentiation, proliferation and apoptosis during both normal development and in human pathologies.

Emerging evidence has demonstrated the dual roles of Notch signaling in cancer formation and progression, which appears to be dependent on cellular context. In medullary thyroid cancer, the absence of Notch signaling directly predicts the expression of downstream markers associated with malignancy, including basic helix-loop-helix (bHLH) transcription factors. Moreover, activation of the Notch1 homolog was found to inhibit these malignant markers and induce growth arrest. In non-neuroendocrine epithelial thyroid cancers, Notch1 was found to be consistent with its predefined tumor suppressor role. Recent evidence has identified Notch3 as also behaving as a tumor suppressor in medullary thyroid cancer, and that its targeted activation causes growth suppression and apoptosis. However, unlike Notch1, Notch3 has yet to be explored in thyroid cancers of follicular origin. These epithelial based thyroid cancers span various degrees of differentiation, from poorly- to well-differentiated. Given Notch3's dynamic expression patterns during development, we sought to better understand the role of Notch3 in the differentiation status of thyroid cancer. Furthermore, we evaluated Notch3 as a prognostic indicator and as a determinant of clinicopathological features among thyroid cancer patients.

DISSERTATION ABSTRACT (cont'd)

We demonstrate that Notch3 correlates positively with the degree of thyroid cancer differentiation, and its absence portends a poor prognosis for patients. Aggressive, poorly-differentiated thyroid cancers expressed low levels of Notch3 while well-differentiated thyroid cancers with better prognoses possessed higher levels. Moreover, normal thyroid tissue and benign thyroid pathologies possessed the highest levels of Notch3 expression. Notch3 expression correlated negatively with tumor size and stage, as well as rates of extrathyroidal extension, distant metastasis, multifocality, and mortality. Specific biomarkers such as Notch3 may help to predict thyroid cancer behavior and could impact the scope of initial surgical therapy. Our discovery of this inverse relationship between Notch3 and thyroid cancer aggression led us hypothesize that restoration of Notch3 in advanced thyroid cancer could curb its aggressiveness by inhibiting its proliferative capacity and inducing re-differentiation. We demonstrated that stably reintroducing Notch3 expression in a follicular thyroid cancer line suppressed cell viability in vitro and reduced tumor burden in vivo through activation of the intrinsic apoptotic cascade. This demonstrates for the first time that Notch3 behaves as a tumor suppressor in follicular based thyroid cancer, and warrants its candidacy as a therapeutic target.

Previous studies have demonstrated the ability of histone deacetylase inhibitors (HDACIs) to activate Notch signaling in thyroid cancer as well as other endocrine malignancies. In addition, these agents have been shown to inhibit cancer cell growth and promote terminal re-differentiation in poorly-differentiated thyroid cancer cell lines.
However, the effect of these agents on Notch3 remains undefined. Given its tumor suppressor role in follicular based thyroid cancer, we postulated that Notch3 would serve as a viable molecular target in these malignancies.
Hence, we aimed to identify select Notch3 activating agents among the HDACI class of pharmacologics, and explore their capacity to reduce thyroid cancer cell proliferation and promote differentiation. Our findings demonstrate that the HDACIs Valproic Acid (VPA), suberoylanilide hydroxamic acid (SAHA), and the exploratory SAHA analog known as AB3, dose dependently reduce thyroid cancer proliferation in both well- and poorly-differentiated thyroid cancer cell lines, while robustly activating Notch3 expression.
Furthermore, these agents were shown to increase transcription of thyroid specific differentiation markers including paired box gene 8 (PAX8) and thyroid transcription factors 1 and 2. These data demonstrate that Notch3 activating therapy may be an effective approach in reducing tumor burden and inducing re-differentiation in advanced thyroid cancer.