VITA

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ABSTRACT

Papillary thyroid cancer (PTC) is the most frequently diagnosed form of thyroid cancer, representing the majority of all cases (13). For unknown reasons it is more commonly diagnosed in women then in men and over the past three decades, its incidence has been rising in the United States (249). It is unclear why this is occurring while the incidence of other cancers is declining (2). Perhaps changes in diagnostic criteria, improvement in early detection and advances in technology are the reasons. However, the overall occurrence of all tumor sizes and the annual number of patients who die from thyroid cancer has also risen (5); contradicting the notion that these reasons alone can account for the entire increase. This fact has prompted thyroid research to focus on investigating the pathways that lead to pathogenesis with a goal to develop new therapeutics. If detected at an early stage, PTC is curable most of the time (16); however, for patients with aggressive and invasive forms of it, treatment options are limited and prognosis is poor (8), (294). Predictors of death from PTC include the presence of gross local invasion and the development of distant metastases (294). Therefore, understanding the mechanisms underlying not only PTC development, but also local invasion and metastases are imperative to develop new therapies targeted for this group of tumors.

In thyroid cancer, the most frequently upregulated signaling cascade is the mitogen-activated protein kinase (MAPK) pathway, which propagates signals from surface receptors to the nucleus via serine-threonine kinases (39). Cell processes that are regulated through this pathway include growth, differentiation, development, inflammation and apoptosis (39). In thyroid cancer, gene rearrangements and point mutations in MAPK signaling molecules are a common event (13). These oncogenic events cause constitutive activation and are generally mutually exclusive within individual tumors (13). These facts have prompted pharmaceutical development to focus on the MAPK pathway, for the past several years (295). Despite these efforts and multiple ongoing studies, to date, there are no effective FDA-approved therapies for patients with progressive metastatic thyroid cancer (8). One particular predictor of aggressive tumor behavior (particularly extensive local
invasion) and unresponsiveness to treatment is the presence of the activating mutation BRAF V600E (296-297).

From our prior studies, we determined that the invasive fronts of aggressive PTC exhibited a characteristic change in morphology known as epithelial-to-mesenchymal transition (EMT) (256). EMT has been linked to aggressive or late stage tumor invasion and metastasis (298). It frequently occurs in PTCs with the BRAFV600E mutation (283); however, EMT has also been identified in its absence, suggesting it is a common endpoint for aggressive PTC behavior. After analyzing the major signaling nodes in the invasive fronts, evidence indicated that there was preferential activation of p21-activated kinase (PAK) signaling (256). When PAKs are activated, they redistribute to the leading edge of a cell and direct the formation of lamellopodia and motility (196-197). In addition, PAK overexpression has been discovered and implicated in the progression of a number of other solid tumors, particularly breast cancer (261), (299).

This data combined with the previous facts, convinced us to focus on investigating the mechanism(s) by which BRAF mutant PTCs acquire an aggressive phenotype. Our hypotheses were that PAKs regulate thyroid cancer cell motility and were involved in BRAF-mediated tumor invasiveness. Before conducting functional studies, we characterized PAK isoform expression in six human thyroid cancer cell lines, normal human thyroid tissue, and thyroid cancer tissue by quantitative RT-PCR and Western blot (WB). All expressed PAKs 1, 2, 3, 4, and in most cases PAK6. A literature search suggested that Group I PAKs were the isoforms most likely involved, so we focused the functional experiments on this group. We transfected into the six thyroid cancer cell lines a group I specific molecular inhibitor called PID. It reduced trans-well filter migration by more than 50% without altering cell viability. To discern which of the three group I PAK isoform(s) were specifically regulating thyroid motility, two of the six cell lines were transfected with PAK 1, 2 or 3-specific siRNA. Only the PAK1 siRNA reduced migration significantly in both cell lines.

To investigate this observation in vivo, we immunohistochemically stained invasive PTCs samples and demonstrated that there was a statistically significant increase in both pPAK and PAK1 expression in the invasive fronts vs. the tumor center, which correlates with the earlier profile studies. Therefore, we concluded...
that PAK isoforms 1-3, 4 and 6 are commonly expressed in human thyroid cancer tissues and cell lines. In particular, PAK1 regulates thyroid cancer cell motility, and pPAK and PAK1 levels are increased in PTC invasive fronts. [(264)]

Now that PAKs had an established role in thyroid cancer progression, we wanted to explore if there was a relationship between PAK activation and the mutational status of BRAF. We selected three human thyroid cancer cell lines (BCPAP, TPC1 and FTC133) with different mutations and transfected in BRAF siRNA. It reduced group I PAK function in all three-cell lines regardless of the mutation, while group I PAK suppression did not affect BRAF function. So we hypothesized that BRAF regulated thyroid cancer cell motility through the activation of group I PAKs. We tested this by performing migration rescue experiments with constitutive active (CA) PAK1, which did rescue the decrease in cell motility induced by the BRAF siRNA. In addition, because MEK1/2 is a well-established BRAF downstream effector, we verified using rescue experiments that it also plays a role in BRAF-mediated migration. We inhibited both MEK1/2 and group I PAKs and showed a lack of synergy. To determine whether one regulated the other, we inhibited MEK1/2 activity and examined PAK function, which was not affected by the suppression. Using both an overexpression and endogenous system, we established that BRAF and PAK1 are co-localized as well as physically bound. 

To further explore this relationship in vivo, thyroids from doxycycline inducible BRAF V600E mice were immunohistochemically stained for pPAK and total group I PAKs. The inducible BRAF V600E mouse thyroids showed an increase in pPAK expression compared to wild-type thyroid controls, with levels of total PAK expression unaltered. In conclusion, we have identified a new signaling cascade in which BRAF activates PAK1 in a MEK-independent manner. This pathway regulates thyroid cancer cell motility and may be an important novel BRAF-regulated pathway involved in thyroid cancer invasion.
RECENT ABSTRACTS AND PRESENTATION

2011 13th OSUCCC-James Annual Scientific Meeting (Poster)
2011 25th Annual Hayes Graduate Research Forum (Oral)
2011 10th Annual OSUMC Trainee Research Day (Poster)
2011 81st Annual American Thyroid Association Meeting (Oral)
2012 11th Annual OSUMC Trainee Research Day (Poster)
2012 26th Annual Hayes Research Forum (not accepted)
2013 27th Annual Hayes Research Forum (not accepted)

RECENT PUBLICATIONS


AWARDS AND HONORS

2011 American Thyroid Association Fellows Travel Grant

FUTURE PLANS

I plan to stay with my current advisor doing a mini postdoc for a year. Then, I will complete a full two to four year postdoc wherever my husband is matched. My future plans include working towards a career in academics.