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Thyroid cancer cell lines: critical models to study thyroid cancer biology and new therapeutic targets

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Thyroid cancer is the most common endocrine malignancy and the incidence is rising. Currently, there are no effective treatments for patients with advanced forms of thyroid cancer. Anaplastic thyroid represents the most severe form of the disease with 95% mortality at 6 months. It is therefore critical to better understand the mechanisms involved in thyroid cancer development and progression in order to develop more effective therapeutic strategies. Cell lines derived from thyroid tumors represent a critical tool to understand the oncogenic mechanisms driving thyroid cancer, as well as preclinical tools to study the efficacy of new therapies *in vitro* and *in vivo*. For thyroid cancer, the development of new therapies has been hampered by the lack of thyroid cancer cell lines in the widely used NCI-60 panel which has been used to screen over 100,000 anti-cancer drugs. In addition, the recent discovery that ~20 out of 40 existing thyroid cancer cell lines are either redundant or misidentified with cell lines of other tissue lineages has further hampered progress in the field. Of the available cell lines, 23 were identified as unique and presumably of thyroid origin based on the expression of thyroid-specific genes. Thus, there is a great need for validated thyroid cancer cell lines representing different stages of disease in addition to distinct oncogenic mutations. New, authenticated thyroid cancer cell lines are beginning to be developed, adding to the tools available to study genes and pathways important for thyroid cancer pathogenesis. In summary, the use of validated thyroid cancer cell lines that closely recapitulate disease is critical for the discovery of new drug targets and ultimately new therapies.

Keywords: thyroid cancer cell lines, cell line misidentification, authenticated thyroid cancer cell lines, ATC, PTC, FTC

INTRODUCTION

There are currently no effective therapies for patients with advanced thyroid cancer, which includes patients diagnosed with advanced papillary thyroid cancer (PTC) and anaplastic thyroid cancer (ATC; Pfister and Fagin, 2008; Smallridge et al., 2009). More than 1300 patients with thyroid cancer die each year from this disease and the incidence is increasing (Leenhardt et al., 2004; Davies and Welch, 2006; Xing, 2008; Enewold et al., 2009). ATC is one of the most aggressive human cancers with greater than 95% mortality at 6 months. Extrathyroidal invasion and metastasis are the most common causes of thyroid cancer-related death, and although much effort has been devoted to decipher the mechanisms involved in the progression of this cancer, little progress has been made in the development of new, effective therapies at this stage (Pfister and Fagin, 2008; Smallridge et al., 2009). Much attention has been devoted to the mitogen-activated protein kinase (MAPK) pathway as a therapeutic target due to the large percentage of activating mutations in this pathway (*BRAF*, *RAS*, *RET/PTC*; Gupta-Abramson et al., 2008), and although these studies are promising, additional therapeutic strategies are needed for the effective long-term treatment of advanced thyroid cancer patients (Pfister and Fagin, 2008).

Cell lines represent a critical tool to study mechanisms driving cancer initiation and progression, and remain an essential preclinical model to test new therapies. Although results using cell lines do not always recapitulate the clinical response, cell lines nonetheless remain an essential tool to test pro-tumorigenic mechanisms and new therapies. Despite the importance of cell lines, recent studies have shown that 18–36% of cell lines are misidentified or cross-contaminated with other cell lines, thus compromising the interpretations of numerous published studies (Masters et al., 2001; Chatterjee, 2007; Lacroix, 2008; American Type Culture Collection Standards Development Organization Workgroup ASN-0002, 2010). Cross-contamination with the HeLa cell line is a well-known historical problem (MacLeod et al., 1999). More recently, cross-contamination of newly developed cell lines with older, established cell lines is becoming an increasingly recognized problem. Indeed, numerous misidentifications have been reported, some within the well-studied NCI-60 panel of cell lines (Lorenzi et al., 2009), in addition to cell lines thought to be of prostate (van Bokhoven et al., 2001a,b, 2003), breast (Rae et al., 2004, 2007; Liscovitch and Ravid, 2007), esophageal (Boonstra et al., 2007), adenoid cystic carcinoma (ACC; Phuchareon et al., 2009; Zhao et al., 2011), and head and neck squamous cell cancer (HNSCC) origin (Zhao et al., 2011).

MISIDENTIFICATION OF THYROID CANCER CELL LINES

In 2008, we reported the comprehensive characterization of a panel of 40 thyroid cancer cell lines (Schweppe et al., 2008). Using genetic profiling, we showed that approximately 40% of these cell

lines were either cross-contaminated with other thyroid cancer cell lines, or misidentified with a cell lines from other tumor types. Specifically, 6 out of 40 cell lines were redundant (identical to other thyroid cancer cell lines), and 10 were misidentified with melanoma or colon cancer cell lines (Schweppe et al., 2008). For these studies, we used short tandem repeat (STR) profiling, which is the internationally approved method for determining cell line identity (Masters et al., 2001). Although STR profiling provides a powerful approach to determine genetic identity, it does not provide information on tissue of origin. Thus, we analyzed expression of the thyroid-specific transcription factors, Pax-8 and TTF-1 to further characterize the origin of these cell lines. For the misidentified cell lines of non-thyroid origin, expression of Pax-8 and TTF-1 were low to undetectable by quantitative real-time RT-PCR (qRT-PCR; unpublished observations). Of the remaining 23 unique cell lines that are likely of thyroid origin, all PTC and FTC cell lines expressed either Pax-8 and/or TTF-1, and about half of the ATC cell lines expressed one or both of these thyroid-specific transcription factors (Schweppe et al., 2008). While expression of Pax-8 and/or TTF-1 do not prove or disprove the origin of these cell lines, the presence of either Pax-8 or TTF-1 supports these cells being of thyroid origin. Critically, none of the unique thyroid cancer cell lines available at the time of this study have been genetically linked to their corresponding tumors, due to the lack of original patient tissue samples. Thus, it is difficult to determine when these misidentifications and cross-contaminations occurred. However, based on the timeline of establishment, it is likely that these cell lines were established by cross-contamination at the time of development (Schweppe et al., 2008). To add to the number of misidentified thyroid cancer cell lines, the ONCO-DG-1 "thyroid cancer" cell line was shown to be a misidentified with an ovarian cancer cell line, the FB2 "thyroid cancer" cell line was shown to be a derivative of the TPC1 cells, and the K1 cell line is likely a derivative of the GLAG66 thyroid cancer cell line (Ribeiro et al., 2008; Schweppe et al., 2008). Thus, it is clear that the development and characterization of new, authenticated thyroid cancer cell lines that are linked to their corresponding tissue samples is needed to complement studies using the existing panel of genetically unique cell lines to accurately study the mechanisms of thyroid cancer pathogenesis, and for the development of new therapeutic strategies.

NEW MODELS TO STUDY THYROID CANCER BIOLOGY

The development of new cancer cell lines is a challenging task with a low success rate, even for more aggressive tumors, and the establishment of cell lines of thyroid origin appears to be a particularly challenging task. Compared to other tumor types, the number of thyroid cancer cell lines is relatively small. The reasons for this low success rate are unclear, but could be partly due to the overall less aggressive nature of these tumors. Of the 784 cancer cell lines in the SANGER database[1], which maintains data for cancer cell lines that are widely available to the scientific community, only 5 of these are thyroid. Similarly, other major databases, including GlaskoSmithKline (GSK), which contains genomic profiling data for >300 cancer cell lines[2], and the Broad-Novartis Cancer Cell Encyclopedia, which has data for 1000 cell lines[3], also only contain a handful of thyroid cancer cell lines. While we identified 23 unique thyroid cancer cell lines, many of these are not available in major repositories. The American Type Culture Collection (ATCC) contains one thyroid cancer cell line of medullary thyroid cancer (MTC) origin, while the German Collection of Microorganisms and Cell Cultures (DSMZ) and European Collection of Cell Cultures (ECACC) contain a handful of genetically unique human thyroid cancer cell lines. Thus, the development of new thyroid cancer cell lines genetically linked to the original tissue sample is needed to improve our understanding of thyroid cancer biology, and once these cell lines are developed, these cell lines should be made available to the scientific community through a repository in order to maintain quality control.

Recently, Marlow et al. (2010) reported the development and characterization of four new ATC cell lines, THJ-11T, THJ-16T, THJ-21T, and THJ-29T. Of importance, STR profiling showed that the newly developed cell lines are genetically unique and match their corresponding tissue samples (Marlow et al., 2010). These THJ cell lines represent the first panel of thyroid cancer cell lines that has been genetically linked to their corresponding tissue of origin, providing a critical new tool to accurately study ATC biology. Interesting, these cell lines exhibited similar patterns of chromosomal losses and gains compared to the original tumor tissue, as determined by array-based comparative genomic hybridization (CGH) analysis, suggesting that minimal genetic drift has occurred in culture (Marlow et al., 2010). The morphology of these cell lines was shown to be squamoid, spindle, giant, or a combination of these morphologies, similar to the original tumor samples (Marlow et al., 2010). Many of the common oncogenic alterations in ATC were also identified in these cell lines, including mutations in *BRAF*, *TP53*, *RB*, *RAS*, and *PI3KCA* (Marlow et al., 2010). Consistent with the ATC origin of these cell lines, rearrangements in RET/PTC and PAX-8/PPARγ were not identified. Oncogenic proteins, including β-catenin, cyclin D1, survivin, and Bcl2 were also evaluated and are expressed at varying levels in the different cell lines, providing new tools to study common oncogenic signaling pathways in thyroid cancer (Marlow et al., 2010). Thyroid-specific gene expression was also evaluated by RT-PCR in this panel of cell lines. Thyroglobulin (Tg), sodium iodide symporter (NIS), and Pax-8 expression was detected in all four cell lines, TTF-1 expression was detected in three of these cell lines, while none of these cell lines expressed thyroid peroxidase (TPO; Marlow et al., 2010). Although TSH receptor (TSHR) expression was detected in one cell line, TSHR was not correctly localized to the cell membrane. All four cell lines were also shown to be tumorigenic in nude mice (Marlow et al., 2010). Thus, the development and characterization of these new ATC cell lines, which appear to closely represent their tumor counterparts, will provide valuable models to study the heterogeneity of ATC biology, and will be important tools to test the efficacy of new therapies using *in vivo* preclinical models.

[1]http://www.sanger.ac.uk

[2]http://array.nci.nih.gov

[3]http://www.broadinstitute.org/ccle/home

Another set of new thyroid cancer cell lines was recently developed from follicular thyroid tumors derived from genetically engineered mice expressing a loss-of-function *Pten* allele and an oncogenic *Kras*, which results in constitutive activation of the phosphatidylinostitol-OH kinase (PI3K) and MAPK pathways, a common event in advanced thyroid cancers (Miller et al., 2009; Dima et al., 2011). Unlike many immortalized cancer cell lines established from advanced tumors, these three mouse cell lines (T683, T691, and T826) were shown to have a relatively normal karyotype, with normal chromosome numbers, and only rearrangements in chromosome 4 for two of the cell lines (Dima et al., 2011). Common genetic alterations relevant to thyroid cancer were also tested. Two of the cell lines exhibited complete loss of the tumor suppressors, p16 and p19ARF, while p53 appeared to be functional (Dima et al., 2011). Consistent with a poorly differentiated phenotype, expression of genes important for thyroid differentiation and function, including *Foxe1*, *Nkx2-1*, *Pax-8*, *Duox1/2*, *Nis*, *Pds*, *TG*, *Tpo*, and *Tshr*, were absent (Dima et al., 2011). Due to constitutive activation of the PI3K and MAPK pathways in these cell lines, inhibition of these pathways with small molecule inhibitors was tested. As expected, inhibition of either pathway alone resulted in partial inhibition of growth, and dual inhibition of both the PI3K and MAPK pathways was more effective, similar to their results using primary cells derived from these tumors (Miller et al., 2009; Dima et al., 2011). Since PI3K can mediate activation of the glycolytic pathway in tumors, lactate production and glycolytic metabolism was also evaluated. Compared to normal thyrocytes, lactate production was higher and cell growth was blocked by a glycolysis inhibitor, suggesting that these FTC-derived cell lines have likely switched to a glycolytic mode, and that inhibition of glycolysis may be a potential new therapy for FTC (Dima et al., 2011). Finally, when implanted into immunocompetent mice, all three cell lines were shown to grow tumors and these mice developed lung metastases (Dima et al., 2011). Thus, these new cell lines represent important new tools to study the mechanisms of PI3K and MAPK signaling, and provide a new model to study FTC tumor growth and metastasis in an *in vivo* immunocompetent preclinical model (Dima et al., 2011).

DEVELOPMENT OF NEW THERAPIES FOR THYROID CANCER

The recent discovery of misidentified thyroid cancer cell lines has certainly hampered the progress in the field (Ringel, 2008; Schweppe et al., 2008). These misidentified cell lines have been widely used in over 300 publications in the last 20 years, and as recently reviewed by Kojic et al. (2011), these misidentified thyroid cancer cell lines, especially DRO90, ARO81, and NPA87, have been used in a significant number of preclinical studies to test new targeted therapies for ATC. Thus, numerous studies using these misidentified cell lines require reinterpretation and validation. The development of new therapies for thyroid cancer patients has also been slowed by the lack of thyroid cancer cell lines in the NCI-60 panel, which has been used to screen >100,000 anticancer agents over the last ∼20 years, and has been widely used to study global gene expression patterns and genomic alterations (Shoemaker, 2006). Despite these problems, as outlined below, progress has been made testing new therapies in validated thyroid cancer cell lines, and overall thyroid cancer cell lines have made major contributions to the understanding of thyroid cancer biology and the identification of new candidate targets for therapy (Kojic et al., 2011).

Many of the oncogenic events contributing to thyroid cancer pathogenesis have been identified. Genetic alterations in components of the MAPK pathway, including *BRAF*, *RAS*, and *RET/PTC* are common in PTC, while mutations in *RAS* and *PPARγ/Pax-8* rearrangements are prevalent in FTC (Knauf and Fagin, 2009; Saji and Ringel, 2010; Xing, 2010; Carlomagno and Santoro, 2011). For ATC, mutations in *BRAF* and *RAS* are common, and many of these cancers harbor genetic alterations in *PI3KCA* and *TP53* along with activating mutations in the MAPK pathway (Knauf and Fagin, 2009; Saji and Ringel, 2010; Xing, 2010; Carlomagno and Santoro, 2011). Recently, a high proportion (∼80%) of metastatic thyroid tumors was shown to exhibit dual activation of the MAPK and PI3K pathways due to oncogenic mutations in *BRAF* and *PIK3CA* or *BRAF* and *AKT1* (Ricarte-Filho et al., 2009). Thus, the development of new therapeutic strategies targeting these pathways is of great interest.

Thyroid cancer cell lines provide an important source to better understand oncogenic signaling mechanisms and to develop new and improved therapies. Of importance, many of the oncogenic alterations found in thyroid cancer, including mutations in *BRAF*, *RAS*, *PI3KCA*, and *RET/PTC1* are represented in currently available thyroid cancer cell lines (Schweppe et al., 2008). While early studies used many of the misidentified cell lines to test agents targeting the MAPK and PI3K signaling pathways, subsequent studies using validated thyroid cancer cell lines have confirmed these results (Xing, 2009). Similar to studies in melanoma, thyroid cancer cell lines harboring a *BRAF V600E* mutation are in general more sensitive to treatment with MKK1/2 and BRAF-specific inhibitors (Solit et al., 2006; Leboeuf et al., 2008; Liu et al., 2009, 2011a; Schweppe et al., 2009a; Salerno et al., 2010; Nucera et al., 2011). Despite the presence of an activating BRAF mutation, clinical studies are indicating that the response of thyroid cancer patients to MAPK-directed therapies is not as promising as melanoma patients. These results are consistent with preclinical studies using cell lines, where Montero-Conde et al. (2011) have shown BRAF-mutant thyroid cancer cell lines are less sensitive to selective BRAF V600E inhibition compared to BRAF-mutant melanoma cell lines. The reason(s) for this differing sensitivity are unclear, but may be due to compensatory upregulation of other pathways, including ErbB3 (Montero-Conde et al., 2011). Alternatively, EGFR signaling was recently shown to promote survival in response to BRAF or MEK1/2 inhibition in BRAF-mutant thyroid and colon cancer cells, but not in melanoma (Prahallad et al., 2012). Although further studies are needed in thyroid cancer, these results suggest that combined inhibition of the MAPK pathway and ErbB2 or EGFR may be beneficial in BRAF-mutant thyroid tumors.

Activation of the PI3K pathway represents another major oncogenic pathway in thyroid cancer. Recent studies targeting the PI3K pathway have shown that thyroid cancer cells with activation of the PI3K pathway are preferentially sensitive to inhibitors of this pathway (Liu et al., 2009, 2011a). Despite these promising results, it is not clear whether inhibition of the PI3K pathway alone will

have sufficient activity in advanced thyroid cancers as single agent therapies. Indeed, recent studies have shown that dual targeting of the MAPK and PI3K pathways may represent a more promising strategy for thyroid tumors that harbor genetic alterations in both pathways (Jin et al., 2009; Liu et al., 2010, 2011b). These studies are consistent with the work from Dr. Di Cristafano's laboratory (discussed above) showing genetic activation of both the MAPK and PI3K pathway is necessary for transformation of the thyroid gland, and that dual inhibition of these pathways is more effective than inhibition of either pathway alone (Miller et al., 2009; Dima et al., 2011). Along with the discovery of co-existing mutations of *BRAF V600E* with *AKT1* or *PIK3CA* mutations in metastatic thyroid lesions (Ricarte-Filho et al., 2009), these studies indicate that dual targeting of the MAPK and PI3K pathways represent a promising therapeutic strategy for patients with advanced thyroid cancer.

Another promising therapeutic strategy for PTC and ATC is targeting tyrosine kinase signaling with tyrosine kinase inhibitors (TKIs). Of these, VEGF family members and its receptors have been shown to play an important role in thyroid cancer, independent of oncogene mutational status (Castellone et al., 2008; O'Neill et al., 2010). Several studies have shown that VEGF and VEGFR2 are overexpressed in thyroid tumors, and that VEGF overexpression is associated with decreased disease-free survival and a poor prognosis. Although initial reports used misidentified cell lines to test the role of VEGFR in PTC and ATC (Kim et al., 2005), recent preclinical studies have shown that treatment with vandetanib (ZD6474), a multi-kinase inhibitor of VEGFR, EGFR, and RET, inhibits proliferation of ATC cells *in vitro*, likely via inhibition of the EGFR receptor, and blocks tumor growth in an *in vivo* orthotopic ATC model, primarily through anti-angiogenic mechanisms which are likely mediated by VEGFR2 (Gule et al., 2011). In support of these preclinical studies, several phase II clinical trials with anti-VEGFR multi-kinase inhibitors are underway, including vandetanib, sunitinib, axitinib, sorafenib, motesanib, and XL-184 with variable, but encouraging results (Castellone et al., 2008; Perez et al., 2011).

The Src–focal adhesion kinase (FAK) tyrosine kinase pathway is another emerging therapeutic target for thyroid cancer (Schweppe et al., 2009b). Src and FAK are multifunctional non-receptor tyrosine kinases that are key regulators of growth, survival, migration, and invasion (Kopetz et al., 2007; Schwock et al., 2010). In one previous study, FAK protein was shown to be overexpressed in a subset of PTC and ATC, but the phosphorylation status of FAK was not examined (Kim et al., 2004). We were the first to show that FAK is phosphorylated in a subset of PTC patient tumor samples (Schweppe et al., 2009b). We further showed that FAK is phosphorylated in a panel of validated thyroid cancer cells, and that the growth and invasion of cells with high phospho-FAK are sensitive to treatment with the Src inhibitor, saracatinib (AZD0530; Schweppe et al., 2009b). Inhibition of growth and invasion was independent of oncogenic mutations in the MAPK pathway, suggesting that the FAK-Src pathway represents another major pro-tumorigenic signaling pathway in thyroid cancer, independent of MAPK signaling. Clinical trials are underway testing Src inhibitors in solid tumors, including BMS-354,825 (dasatinib; Bristol-Myers Squibb), bosutinib (SKI-606, Wyeth; Quintas-Cardama et al., 2007), and the more selective Src inhibitor, AZD0530 (saracatinib; AstraZeneca; Hennequin et al., 2006; Santini et al., 2010). Recently, FAK inhibitors have been developed, and two ATP-dependent small molecule inhibitors, including NVP-TAE-226 and the more selective PF-562,271, have entered clinical trials (Halder et al., 2007; Roberts et al., 2008; Siu et al., 2008; Schwock et al., 2010). Thus, future studies testing the efficacy of Src and FAK inhibitors in advanced thyroid cancer will be of great interest.

CONCLUDING REMARKS

Progress is being made in the development and characterization of new thyroid cancer cell lines. The majority of currently available cell lines represent PTC and ATC, while FTC-derived cell lines remain underrepresented. Importantly, while the major oncogenic mutations found in thyroid tumors are represented in many of the available cell lines (*BRAF*, *RAS*, *PI3KCA*, *RET/PTC1*), some of the less common genetic alterations, including *AKT1*, which is likely important in metastatic thyroid cancer (Ricarte-Filho et al., 2009), specific RET/PTC isoforms, which play distinct roles in PTC pathogenesis, and PPARγ/Pax-8 rearrangements, which are important in FTC, are not represented in the current panel of cell lines (Schweppe et al., 2008). For further translational relevance, studies with permanent cell lines should be complemented with studies using human tissue samples, and when possible, primary culture models, which may better recapitulate the original tumor. In conclusion, the continued development and characterization of new cell line models that are genetically linked to the original patient tissue samples is critical to further understand the oncogenic properties of thyroid cancer cells, identify novel targets for therapy, and to translate these findings to the clinic for patients with advanced thyroid cancer.

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