Establishment and Characterization of Novel Human Primary and Metastatic Anaplastic Thyroid Cancer Cell Lines and Their Genomic Evolution Over a Year as a Primagraft


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Context: Anaplastic thyroid cancer (ATC) has no effective treatment, resulting in a high rate of mortality. We established cell lines from a primary ATC and its lymph node metastasis, and investigated the molecular factors and genomic changes associated with tumor growth.

Objective: The aim of the study was to understand the molecular and genomic changes of highly aggressive ATC and its clonal evolution to develop rational therapies.

Design: We established unique cell lines from primary (OGK-P) and metastatic (OGK-M) ATC specimen, as well as primagraft from the metastatic ATC, which was serially xeno-transplanted for more than 1 year in NOD scid gamma mice were established. These cell lines and primagraft were used as tools to examine gene expression, copy number changes, and somatic mutations using RNA array, SNP Chip, and whole exome sequencing.

Results: Mice carrying sc (OGK-P and OGK-M) tumors developed splenomegaly and neutrophilia with high expression of cytokines including CSF1, CSF2, CSF3, IL-1β, and IL-6. Levels of HIF-1α and its targeted genes were also elevated in these tumors. The treatment of tumor carrying mice with Bevacizumab effectively decreased tumor growth, macrophage infiltration, and peripheral WBCs. SNP chip analysis showed homozygous deletion of exons 3–22 of the PARD3 gene in the cells. Forced expression of PARD3 decreased cell proliferation, motility, and invasiveness, restores cell-cell contacts and enhanced cell adhesion. Next generation exome sequencing identified the somatic changes present in the primary, metastatic, and primagraft tumors demonstrating evolution of the mutational signature over the year of passage in vivo.

Conclusion: To our knowledge, we established the first paired human primary and metastatic ATC cell lines offering unique possibilities for comparative functional investigations in vitro and in vivo. Our exome sequencing also identified novel mutations, as well as clonal evolution in both the metastasis and primagraft. (J Clin Endocrinol Metab 100: 725–735, 2015)

Abbreviations: ANCT, adjacent noncancerous tissue; ATC, anaplastic thyroid carcinoma; PARD3, par-3 partitioning defective 3 homolog (C. elegans); PAX8, paired box gene 8; SNP, single nucleotide polymorphism; TG, thyroglobulin; TTF1, transcription factor 1; VAFs, variant allele frequencies; VEGF, vascular endothelial growth factor; VEGFA, vascular endothelial growth factor A; WBC, white blood cells; WES, whole exome sequencing; ZO-1, Zonula occludens 1.
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ey year in the United States, approximately 38 000
 patients are diagnosed with thyroid carcinoma re-
 presenting approximately 1% of all human malignant dis-
 eases (1). Most thyroid carcinoma patients have differen-
tiated tumors which are amenable to therapy. Anaplastic
tumor cancer (ATC) accounts for less than 2% of all the
thyroid malignancies, with a grave prognosis (2). These
tumors are extremely aggressive, and are resistant to ra-
 diation therapy and conventional chemotherapy. Indivi-
duals with ATC have an average 5-year survival rate of
<10%, with a median survival of 4–6 months (3). Novel
effective approaches are clearly needed for the treatment
of ATCs, including targeted therapy.

Establishment and careful analysis of ATC cell lines
should provide the foundation for these advancements.
RNA array and candidate gene studies have identified ge-
etic alterations in RET, p53, RAS, BRAF, and β-catenin
genes in ATC (4). These studies begin to bring into focus
the therapeutic targets of ATC. Next generation sequenc-
ing allows the comparison of genomic changes in multiple
samples taken from the same individual to delineate the
genetic basis for tumor progression and metastasis (5).

We established both primary and metastatic ATC cell
lines from the same patient and also a primagraft which
was serially passaged for > 1 year in NSG mice without in
vitro culture. Using RNA expression arrays, single nucle-
otide polymorphism (SNP) chip analysis and deep exome
sequencing, we identified novel genomic abnormalities
and potential treatment targets for ATC. Differential mu-
tational frequencies in the metastasis and primagraft com-
pared with the primary tumor suggest that secondary tu-
mors may arise from a minority of the cells within the
primary tumor.

Materials and Methods

Patient history

A 71-year-old Caucasian male was diagnosed with a multi-
nodular goiter at age of 62. He underwent multiple thyroid bi-
opseys, which demonstrated benign adenomatous nodules. At
the age of 67, he underwent additional biopsies because of the
growth of one nodule. At age 70, the patient developed prostate
cancer and underwent a prostatectomy and pelvic lymphadenec-
tomy. The lymph nodes in the region of the prostate were free of
disease. During a staging evaluation at the age of 71, multiple pulmo-
nary nodules were found, that had not been present on a
chest CT scan performed 6 months earlier. A PET/CT scan dem-
onstrated multiple PET-positive lung lesions, neck nodes, and a
4 × 6 cm mass in the thyroid gland. A fine needle aspiration of
the thyroid mass showed poorly differentiated malignant cells.
His white blood cell (WBC) count was elevated at 20 000/μL
(normal 4000–11 000/μL) with 81% PMN and an absolute
PMN count of 16 400/μL. He underwent a thyroidectomy and
central compartment lymph node dissection, which revealed a
3.5 cm ATC that was locally invasive and which also involved
two of the 21 lymph nodes. Pathological staging of ATC was
pT4bN1aM1. Immunohistochemistry showed the absence of
the expression of S-100 and TTF-1 and the presence of keratin
(AE1/AE3). The patient died 3 months following surgery. The
diagnosis was established on commonly accepted clinical, labo-
atory, and histological criteria at the Cedars-Sinai Medical Cen-
ter. The study subject gave his informed consent for our study
(IRB # Pro00006795).

Establishment of ATC cell line

Thyroid tumor and lymph node were obtained at surgery
from the patient and immediately processed. Specimens were
minced into small pieces and applied gently against a 40 μm
filter. The filter was washed with RPMI media containing 10%
FBS, and the flow-through was collected by centrifugation.
The pellets were plated on culture dishes. In vitro cell lines were es-

tablished from the primary thyroid tumor (OGK-P) and the met-
astatic lymph node (OGK-M). In addition, filtered cells from the
lymph node were directly inoculated into the peritoneal cavity
of NSG mice without in vitro culture. Forty-two days after the
initial seeding, the cavity was opened and clumps of cells were
harvested and divided into two halves. One half was cultured on
do
dishes as the first generation of OGK-NSG cells; the other half
was cultured without in vitro culture. Forty-two days after the

Statistical analysis

For in vitro and in vivo experiments, we evaluated the statistical
significance of the difference between two groups by the two-tailed
Student t-test and two-way ANOVA. Asterisks in the figures indi-
cate significant differences of experimental groups in comparison to
controls (* P < .05, ** P < .01, *** P < .001). Data points in figures
represent the means ± SD.

Results

Establishment of novel ATC cell lines

Surgical specimens from the primary anaplastic thyroid
cancer and lymph node metastasis were established in tissue

culture (OGK-P and OGK-M, respectively). Cell lines were
considered to be established after 15 passages. Cell lines have
maintained an in vitro culture for more than 36 months (3
years). Doubling time of the OGK-P and OGK-M is 23 and
18 h, respectively, as measured by the online doubling time
calculator (Supplemental Table 1). The metastatic ATC tis-

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(OGK-primagraft), which was serial passaged (19 times) for over 1 year in these NSG mice (Figure 1A). For the initial primagraft (first primagraft), $1 \times 10^5$ cells from patient lymph-node were injected into the peritoneal cavity of NSG mice without in vitro culture. On day 42, the tumor volume was 739.2 mm$^3$ (largest and smallest diameter of the tumor was 20 and 8.4 cm). The primagrafts were serially passaged every month for one and half years. No metastasis was observed in the lungs, liver, and spleen of the tumor bearing mice, but the Institutional Animal Care and Use Committee (IACUC) policies did not allow primagrafts to grow more than 1.5 cm.

**Features of the ATC cells and the primagrafts**

STR profiling data showed that OGK-P, OGK-M, OGK-primagraft, and patient DNA are almost identical (Supplemental Table 2), thus genetically linking the cell lines and primagraft to the patient. Microarray analysis showed a total of 389 genes with twofold expression unique to either OGK-M cells and/or OGK-primagraft compared to OGK-P (Figure 1B, Supplemental Table 10). SNP chip analysis of the OGK-P (Supplemental Figure 1A), OGK-M cell lines (Supplemental Figure 1B), and OGK-primagraft (Supplemental Figure 1C) showed predominantly a trisomy of many chromosomes as summarized in Supplemental Table 3. Of note, two regions of all three samples had loss of one arm of chromosome 10p and 17p and duplication of the other arm. Concerning 17p, loss of one chromosomal arm and duplication of the other resulted in a homozygous mutation of the TP53 gene. Focal homozygous deletion at 10p11.21 containing the PARD3 gene occurred in these cell lines.

OGK-P and OGK-M cell lines were injected sc in athymic nude mice; large tumors were evident within 2 weeks with prominent blood vessels (Figure 1C). By the 28th day, peripheral WBC count, especially neutrophils, markedly increased (> fivefold) in these tumor bearing mice (Supplemental Table 4). These mice developed ~ two- to three-fold enlargements of their spleens (Figure 1D) with my...
eloid metaplasia, and no tumor cells were observed in the spleens. Immunohistochemistry of xenograft tumors showed positive staining for alpha smooth muscle actin (SMA), vimentin, keratin, and mucin 1 (Supplemental Table 5). The OGK-P and OGK-M cell lines, xenografts, and OGK-primagraft cells were examined for thyroid specific genes, such as, the transcription factor 1 (TTF1), thyroglobulin (TG), and paired box gene 8 (PAX8). Immunohistochemistry showed that these were strong (nuclear staining) for PAX8, but negative for TTF1 and TG (18, 19) (Figure 1E, Supplemental Table 5). Both TG and TFF1 have been reported to be strongly expressed in normal thyroid tissue and often completely lost in ATC (20). The xenograft tumors were markedly infiltrated with neutrophils (Myeloperoxidase-positive) and macrophages (F4/80-positive) (Supplemental Table 5).

**Cytokine and growth factor production in ATC cells**

Since splenomegaly and blood neutrophilia developed in the mice carrying both the cell line explants and primagrafts, expression levels of cytokines were measured in these tumors. Relative mRNA expression of CSF1, CSF2, CSF3, IL-1β, IL-6, and IL-7 was higher in OGK-P, OGK-M, and OGK-primagraft as well as other ATC cell lines including HTH83, SW1736, HTH7, T241, T351 cells (measured by real time PCR) (Figure 1F and Supplemental Table 6) compared to BHP2–7 cells (papillary thyroid cancer) and normal thyroid cells. Both qPCR and microarray data revealed that expression of cytokines and their receptors were higher in metastatic cells compared to primary cells. In addition, committed myeloid stem cells from wild-type murine bone marrow proliferated and differentiated in methylcellulose into neutrophils and macrophages when cultured in 10% conditioned media from OGK-M cells consistent with hematopoietic cytokines present in the conditioned media (Figure 1G).

**Bevacizumab treatment of mice carrying the ATC xenograft tumors (OGK-M)**

Both real time quantitative PCR (qPCR) and Western blot data showed higher expression of VEGFA, both at the transcriptional and protein levels in ATC cell lines and patient samples compared to adjacent noncancerous tissue (ANCT) (Figure 2A). Moreover, we observed relatively higher expression of VEGFA in OGK-P cells compared to OGK-M and OGK-primagraft (Figure 2A and Supplemental Table 7). Bevacizumab is a recombinant humanized monoclonal antibody, which suppresses angiogenesis by binding and inhibiting vascular endothelial growth factor (VEGF) A (VEGFA). Bevacizumab is an FDA approved antibody for treatment of several malignancies, such as glioblastoma and metastatic colorectal, lung, renal, ovarian, and breast cancers (21, 22). Bevacizumab was given every other day intraperitoneally (IP) to mice carrying OGK-M xenografts. The antibody prevented tumor growth in the mice who received the therapy starting 1 day after tumor inoculation (Supplemental Figure 2, A and B). Peripheral blood WBC, neutrophils, eosinophils, and basophils in the Bevacizumab-treated group were significantly lower than the tumor bearing control mice receiving diluent. These latter mice had much larger tumors (Supplemental Figure 2, C and D). In another series of experiments, ATC xenografts were grown for 14 days before beginning Bevacizumab treatment (5 mg/kg, three times a week for five courses). These mice treated with Bevacizumab showed significantly decreased tumor volumes ($P < .001$) (mean tumor volume $150.3 \pm 48.0$ mm$^3$ compared with control 300.0 $\pm$ 86.3 mm$^3$) (Figure 2B). Tumor weights of Bevacizumab-treated mice also decreased compared to the control group (Figure 2, C and D). Tumors in the control group had a very vascular capsule surrounding the tumor (Figure 2D). These control mice also had significant ($P < .001$) weight loss (Figure 2E) and elevated WBC counts (Figure 2F) compared to Bevacizumab-treated mice. Tumor infiltration with F4/80 positive cells (macrophages) was significantly greater in control tumors compared to Bevacizumab treated tumors (Figure 2G).

**PARD3 gene is homozygously deleted, and forced expression of PARD3 reduced cellular growth, motility, and invasion, as well as cell-cell junctions**

Homozygous deletion of the PARD3 gene (chromosome 10p11.21) was detected in OGK-P and OGK-M cell lines and OGK-primagraft by analyzing their SNP chip data (Figure 3A). For validation, all exons of PARD3 were examined using PCR primers spanning each exon using genomic DNA of OGK-P, OGK-M cell lines and OGK-primagraft. No PCR products of the PARD3 gene were observed between exons 3 and exon 22 for all three cell sources (Figure 3B). M4A4 cells (wild-type for PARD3) and T98G (PARD3 homozygous deletion between exon 3 and exon 21) (23) were used as controls. In addition, RT-PCR and nucleotide sequencing of OGK-P and OGK-M cell lines and OGK-primagraft showed an absence of wild-type PARD3 mRNA and fusion of the deletion-flanking exons (Figure 3, C and D). PARD3 protein expression was not detected in the OGK-P, OGK-M, OGK-primagraft, and T98G cells (negative control) by Western blot (Figure 3E). Interestingly, five of eight ATC cell lines had very low expression of PARD3 compared to M4A4 (wild type for PARD3, positive control) and the papillary thyroid cancer cell line (BHP2–7) (Figure 3E). We analyzed the TCGA thyroid data, composed of 486 thyroid cancer samples. A
total of 19.13% (94/486) of the thyroid carcinoma samples had down-regulation of PARD3 mRNA and one sample contained a missense mutation (L51W) of the gene (Supplemental Figure 5). Taken together, these observations suggest that during thyroid carcinogenesis a decreased expression of PARD3 often occurs.

Stable clones of OGK-M cells expressing either a PARD3 expression vector or empty vector were established. Ectopic expression levels of PARD3 protein in OGK-M were very similar to PARD3 endogenous levels present in BHP2–7 cells (wild type PARD3) (Figure 4A), suggesting that our forced expression of PARD3 clones were near physiological levels. PARD3 expressing cells had significantly ($P < .001$) slower growth compared to control cells (Figure 4B) and acquired clear cell-cell junctions and a tile structure in culture, while OGK-M cells with an empty vector grew more independently of each other (Figure 4C). Zonula occludens 1 (ZO-1, a marker of cellular tight junctions) was noted on the cell-cell junctions of OGK-M cells stably expressing PARD3, whereas no staining was observed with an empty vector (Figure 4D). Moreover, forced expression of PARD3 significantly ($P < .001$) suppressed motility and invasiveness of OGK-M cells compared to those without PARD3 (Figure 4, E and F).

During cell passage, we observed that OGK-M cells with stably forced expression of PARD3 displayed a strong attachment to the tissue culture plates, requiring longer duration to release the cells from the plates compared to empty vector control cells. We, therefore, considered that PARD3 enhanced cell adhesion. To further explore this possibility,
ECM cell adhesion assays were performed on plates pre-coated with collagen type I and fibronectin. As anticipated, the OGK-M cells with ectopic expression of PARD3 showed significantly increased adhesion to the components of extracellular matrix collagen type I (Figure 4G) and fibronectin as compared to empty vector containing cells (Figure 4H). Together, these results suggest that PARD3 acts as a tumor suppressor gene in thyroid cells.

Identification and evolution of coding mutations by whole exome sequencing (WES) of primary and metastatic ATC samples and continuous > 1 year passaged ATC primagraft

WES was performed on genomic DNAs obtained from primary (thyroid) and metastatic (lymph-node) ATC, 1 year primagraft, and germline control from the same patient. Average nucleotide coverage was approximately 164×, and 84% of all targeted bases were read at least 20 times, sufficient for variant calls (Figure 5A and Supplemental Table 8). The ratio of nonsynonymous to synonymous mutations was 1:2, consistent with the positive selection that typically occurs with driver mutations. A total of 44 nonsynonymous substitutions and 6 indels affecting the integrity of the open reading frames (ORF) were identified in these three tumors. Sanger sequencing validated 39 (true positive rate = 89.13%) of the nonsynonymous SNVs, and 4 indels (true positive rate = 66.7%) were confirmed. Two SNVs could not be tested because of PCR failure (Supplemental Table 9). TP53 (C277Y) mutation was noted in each of these samples.
A total of 43 somatic mutations in primary, metastatic, and primagraft cells were verified using Sanger sequencing, a mutation rate comparable to those of other solid tumors (24). The total number of somatic mutations was higher in the metastatic tumor and primagraft compared to the primary tumor (Figure 5B). Similar to the other cancers, C/T transitions were the most common mutations found in primary and metastatic tumor cells and the primagraft (Figure 5C). Moreover, an increase in A.T > C.G and C.G > G.C transversions occurred in the metastatic tumors and primagraft (Figure 5C). Notably, an increased A.T > C.G transversion rate has also been observed in cases of chronic lymphocytic leukemia (25).

Homozygous TP53 mutations were present in the primary and metastatic ATC samples and primagraft cells. Both PLCD4 (R325Q) and NFE2L2 (W8G) missense mutations progressed from being heterozygous in the primary and metastatic ATC to homozgyously deleted in the primagraft. Also, the PDIA6 (A339G) gene was heterozygously mutated in the primary ATC, and homozygously mutated in the metastatic ATC and the primagraft (Figure 5D, Supplemental Figure 3). The SIFT test was used for the prediction of damaging mutations, and most of the validated mutation scores indicated that those on the list were damaging mutations (Supplemental Table 9). DNMT1 and SMG1 genes were only mutated in the primagraft.

The variant allele frequencies (VAFs) for somatic mutations validated in each tumor sample were determined by using WES data (mean depth of 164 reads). Based on the VAF distribution, the number and size of the clonal populations in each tumor sample was calculated. Based on mutation clustering results, 3 clones were observed with a distinct set of mutations in the primary tumor. The median mutant allele frequencies in the primary tumors
for cluster 1–3 were 42.3%, 39.13%, 18% (Figure 6, A and C). Cluster 1 is the “founder” since the other subclones were derived from it. We assume that almost all of these mutations are heterozygous with a variant allele frequency of 40–50% and must be present virtually in all the tumor cells of the primary and metastasis (Figure 6D). Clusters 2 and 3 mutations must be derived from clone 1. A single clone containing all of the cluster 4 mutations was detected in the metastatic sample. Clone 5 evolved from clone 4, but gained additional mutations. However, in the metastasis and primagraft, three mutant clones were noted. Clone 1 represents the “founder” which is present in both the metastatic (clone 2) and primagraft clones (clone 3) (Figure 6D). Clone 4 mutations were present both in the metastatic tumor and primagraft, but were absent in the primary tumor (Figure 6, B-D). Taken together, progression of the disease was accompanied by acquiring additional somatic mutations.

**Discussion**

ATC is a rare malignancy, but extremely aggressive and resistant to chemotherapy (26). Cell line model systems have made significant contributions to cancer research. Most cancer cell lines are from either primary or metastatic tumors. In this study, we have established, to our knowledge, the first matching human ATC cell lines from the primary (OGK-P) and the metastatic lymph node (OGK-M). In addition, a primagraft was established directly from the metastatic lymph-node which has been serially passaged for more than 1 year in NSG mice (OGK-
The primagraft has never been grown in vitro.

Our newly established cell lines formed aggressive tumors in athymic nude mice and robustly recruited blood vessel formation. In addition, mice carrying these cells developed splenomegaly containing increased myeloid cells, but no tumor cells. Furthermore, the xenografts were markedly infiltrated with neutrophils and macrophages suggesting the tumor cells were producing chemokines to stimulate the migration of hematopoietic cells to the tumors. Our microarray and qPCR data revealed that our established ATC lines had very high expression of various cytokines. Furthermore, condition media collected from these ATC cells stimulated clonogenic growth of murine hematopoietic committed stem cells to form granulocyte and macrophage colonies. Of note, we also performed IHC on the patient’s thyroid tumor and lymph node metastasis and found rich invasion with neutrophils and macrophages (Supplemental Figure 4, A and B). Previously, Fagin’s group (27) as well as others (28) have noted hematopoietic cell invasion into ATC. Recently, investigators working with many different model systems using a variety of tumors have posited that these invading hematopoietic cells enhance the growth of the tumors (29). Here, we showed that the primary and metastatic ATC cells produce many types of cytokines, which probably explains both the rich invasion of hematopoietic cells into the tumor, as well as the leukocytosis found in the patient.

qPCR and Western blot data showed high expression of VEGF-A in ATC patient samples, ATC cell lines (OGK-P, OGK-M, OGK-primagraft, SW1736, HTH7, T238, CAL62) compared to adjacent noncancerous tissue. Humanized monoclonal antibody, Bevacizumab, binds and inhibits VEGF-A, and is FDA approved for treatment of various cancers including colorectal, lung, renal tumors, as well as gliomas. Bevacizumab in combination with doxorubicin is in phase two clinical trial for ATC patients. Treatment with Bevacizumab in mice carrying OGK-M xenografts significantly decreased their tumor growth and weight; and the mice tolerated the therapy with no apparent side effects (eg, weight loss).

Figure 6. Clonal evolution of the primary and metastatic ATC samples and the > 1 year continuous passaged ATC primagraft and graphical representation of clonal evolution from primary to metastatic to primagraft. A and B, Diagonal plots showed the distribution of variant allele frequencies (VAF) of validated mutations listed in Supplemental Table 9 in both primary and metastasis (A); and in both metastasis and primagraft (B), where VAFs of genes in the region of uniparental disomy (UPD) were halved. Driver mutations including TP53 are indicated by red arrows. In A, the founding clones in the primary and metastatic tumor are represented by blue, whereas pink and orange represent metastasis specific clones. Similarly in B, green represented the primagraft specific clone. C, Mutation clusters observed in the primary, metastatic, and primagraft tumors. Relationship between clusters in the primary, metastasis, and primagraft are indicated by linking the lines between them. D, The “founder” clone in the primary tumor contained somatic mutation in the TP53 gene. The dominant clones in the primary ATC tumor evolved into the metastatic clone by acquiring metastatic specific mutations. One of the clones in the metastasis (founder) also emerged as a founder clone in the primagraft. Another dominant clone at metastasis remained in the primagraft clone; also a primagraft specific clone emerged.
High density SNP arrays noted that OGK-P, OGK-M, and OGK-primagraft cells had a small homozygous deletion at chromosome 10p11.21 encompassing exons 3–22 of the PARD3 gene. PARD3 is a master regulator of apical-basal cell polarity, a process that has been indirectly implicated in tumorogenesis (30, 31). OGK-P, OGK-M, OGK-primagraft, HTH7, C643, and T241 ATC cells had either undetectable or very low protein expression levels of PARD3. Furthermore, force-expression of PARD3 in OGK-M cells suppressed their cellular proliferation, motility, invasion, and enhanced the formation of cell-cell junctions, as well as cell adhesion to the collagen type I and fibronectin. Taken together, PARD3 behaves as a tumor suppressor in thyroid cells, and ATC cells lose expression of the gene to enhance their invasive features.

Next-generation sequencing (NGS) technology enhances scientific knowledge and aid in making clinical decisions. NGS can even be performed on formalin-fixed and paraffin embedded tissue (32). To our knowledge, this is the first report of WES of a primary and metastatic ATC, as well as comparing these results to the metastatic tumor passedaged in NSG mice for > 1 year (primagraft). All mutations were validated by a second technique (Sanger sequencing). Burden of mutations was higher in the metastatic tumor and primagraft compared to the primary ATC tumor. Also, C > T and G > A transitions and A.T > C,G and C.G > G.C transversions were the most frequent mutations in these three tumor types, but their frequency varied between them. Using a variety of software (SIFT, PolyPhene), meaningful driver mutations were identified. A TP53 homozygous mutation was verified in the primary and metastatic tumors, as well as the primagraft passedaged for > 1 year. TP53 mutations have been associated with high levels of VEGF. Treatment of these mice with Bevacizumab markedly decreased the growth of these tumors recruiting copious blood vessels associated with high levels of VEGF. Treatment of these mice with Bevacizumab markedly decreased the growth of these tumors. Nevertheless, our data showed that the “founder” clone was present in the primagraft, indicating that they evolved to a distinct subset of the mutational repertoire of the primary tumor.

Recent studies noted the importance of clonal evolution in tumor progression and development of metastasis (34, 35). Our data displayed a wide range of mutant allele frequencies suggesting considerable genetic heterogeneity in the cellular population of the primary tumor. The range of mutational frequencies narrowed in the metastasis and primagraft, indicating that they evolved to a distinct subset of the mutational repertoire of the primary tumor.

Several commercial companies are advertising the therapeutic discriminatory power of growing primagrafts of the patient’s tumors, sequencing the primagraft to identify targetable mutations followed by treating the tumor-bearing mice with selective drugs. We found that primary and metastatic ATC samples contained 11 mutated genes not present in the primagraft. Furthermore, the primagraft after 20 passages over 19 months had 14 mutant genes not present in either the primary or metastatic lesions. We do not know if these primagraft specific mutations were also present at an “undetectable” frequency in the primary and metastatic tumors. At this stage, our data suggest some caution in the acceptance of a proposed therapy as suggested by primagraft analysis because the driver mutations may have developed in the primagraft independent of its development in the primary tumors. Nevertheless, our data showed that the “founder” clone was present in the primagraft thus testing for drugs directed against the “founder” mutant gene may provide a therapeutic benefit.

In summary, we developed a matched pair of primary and metastatic aggressive ATC cell lines that easily form xenografts recruiting copious blood vessels associated with high levels of VEGF. Treatment of these mice with Bevacizumab markedly decreased the growth of these tumors. The SNP array showed homozygous deletion of PARD3; re-expression of PARD3 inhibited ATC proliferation, motility and renewed the normal tiled epithelial pattern. Deep nucleotide sequencing of the primary, metastasis and primagrafts suggest that new somatic mutations do occur during the clinical course of the disease, but most of the original “founder” mutations present in the primary tumor were also present in the metastasis and primagraft, suggesting that early primagrafts are valid for therapeutic discriminatory power of growing primagrafts of the patient’s tumors, sequencing the primagraft to identify targetable mutations followed by treating the tumor-bearing mice with selective drugs. We found that primary and metastatic ATC samples contained 11 mutated genes not present in the primagraft. Furthermore, the primagraft after 20 passages over 19 months had 14 mutant genes not present in either the primary or metastatic lesions. We do not know if these primagraft specific mutations were also present at an “undetectable” frequency in the primary and metastatic tumors. At this stage, our data suggest some caution in the acceptance of a proposed therapy as suggested by primagraft analysis because the driver mutations may have developed in the primagraft independent of its development in the primary tumors. Nevertheless, our data showed that the “founder” clone was present in the primagraft thus testing for drugs directed against the “founder” mutant gene may provide a therapeutic benefit.

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these primagrafts, which may differ from the mutational landscape of the patient’s primary tumor.

**Acknowledgments**

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This work was funded by the Singapore Ministry of Health’s National Medical Research Council under its Singapore Translational Research (STAR) Investigator Award to H. Phillip Koehler (National Research Foundation Singapore and the Singapore Translational Research (STaR) Investigator Award to H. Phillip Koehler) and NIH Grant No. 2R01CA026038-35. Generous support also comes from the Thyroid Centre of Excellence, Cedars Sinai Medical Center.

Disclosure Summary: The authors have nothing to disclose.

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