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Metastatic Mechanisms in Follicular Cell-Derived Thyroid Cancer

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Abstract

Thyroid cancer incidence is rising annually largely related to enhanced detection and of early stage well-differentiated primary tumors. The prognosis for patients with early stage thyroid cancer is outstanding with most patients being cured with surgery. In selected cases, I-131 is administered to treat known or suspected residual or metastatic disease. Even patients with loco-regional metastases typically have an outstanding long-term prognosis, albeit with monitoring and occasional intervention for residual or recurrent disease. In contrast, individuals with distant metastases from thyroid cancer, particular older patients with larger metastatic burdens and those with poorly differentiated tumors, have a poor prognosis. Patients with metastatic anaplastic thyroid cancer have a particularly poor prognosis. Published clinical trials indicate that transient disease control and partial remissions can be achieved with kinase inhibitor therapy directed toward angiogenic targets, and that in some cases, I-131 uptake can be enhanced. However, the direct targets of activity in metastatic lesions are incompletely defined and clear evidence that these treatments increase the duration or quality of life of patients is lacking, underscoring the need for improved knowledge regarding the metastatic process to inform the development of new therapies. In this review, we will focus on current data and hypotheses regarding key regulators of metastatic dormancy, metastatic progression, and the role of putative cancer stem cells.

Keywords

Metastases; Thyroid Carcinoma; Metastases Suppressors; Angiogenesis; Tumor Stem Cells

INTRODUCTION

Metastatic disease is the primary cause of cancer mortality for most solid tumors including thyroid cancer (Kitamura, et al. 1999; Mazzaferri and Kloos 2001). Distant metastatic

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disease is present at presentation in only 3 – 15% of patients with thyroid cancer, but develops later in 6 -20% of patients (Nixon, et al. 2012). The concept of "targeted therapy" has been used in the treatment of metastatic thyroid cancer for decades. Specifically, thyroid hormone is often administered at doses sufficient to reduce circulating levels of thyrotropin (TSH) to inhibit growth of thyroid cancer cells that express the TSH receptor, and radioiodine (I-131) may be used to deliver ionizing radiation with relative specificity to thyroid cancer cells based on expression of the Na, I symporter (NIS). Although TSH-suppression and I-131 treatment improve outcomes in patients with stage 3 or 4 thyroid cancer, the absence of uptake or response to therapy occurs commonly and can be a harbinger of progressive disease. For example, a large study of patients with metastatic thyroid cancer found that patients who have iodine avid and responsive metastatic thyroid cancer have a 10-year survival rate of 90%, while those with I-131 non-avid thyroid cancer have a 10-year survival rate of only 10% (Durante, et al. 2006). A more recent study demonstrates the overall lack of oncologic benefit in the majority of patients treated with I-131 for distant metastases (Sabra, et al. 2013). These studies emphasize that I-131 therapy when administered as a solitary therapy is rarely curative in patients with thyroid cancer. A better understanding of the mechanism of metastasis and subsequent late stage progression in metastatic microenvironments has potential to improve therapeutic targeting for patients with aggressive thyroid cancer.

More than 100 years ago, in an attempt to explain the organ specific pattern of metastatic cancer, Paget first proposed the "seed and soil" hypothesis of cancer metastases (Paget 1889). He speculated that the ability of metastatic lesions to occur and progress depended both on the nature of the primary cancer cells and the environments to which they are exposed. Forty years later, Ewing challenged the theory by proposing that metastatic spread is purely due to mechanical factors of the vascular anatomy. This idea became a major alternative theory for years, but multiple researchers have since shown this is not the case. Not only are there differences in the endothelial cells comprising capillaries in different organs (Trepel, et al. 2002), but there is a very complex interaction between the microenvironment of the host and the cancer cells.

Metastasis is comprised of a series of complex steps that ultimately result in the presence of growing tumor implants in non-native locations. In the classical model of metastatic progression, it has been proposed that the steps in the metastatic cascade are sequential: The tumor cell must detach from the primary tumor, intravasate into the vascular system, survive its voyage through the circulatory system, arrive within the vasculature of an organ, extravasate from the vascular system, survive, and then proliferate in the target tissue. Large primary tumors have been shown to lose millions of tumor cells into the vasculature daily, but only a few eventually become distant metastases. From a cancer biology perspective of this model, it is proposed that cells undergo a multistep process of mutations that result in progressive dedifferentiation, eventually leading to epithelial-to-mesenchymal transition when they develop the ability to invade into local structures including blood vessels with capacity to form metastatic foci at a distant site. After implantation, the cells develop a new vascular supply and subsequently proliferate. While this series of events has been viewed as a late event in the progression of cancer, there is now evidence to support that metastasis can occur earlier in some tumor types (Husemann, et al. 2008) or as a late event and may not

require dedifferentiation at the primary tumor site (Yachida, et al. 2010). Further complicating the traditional step-wise progression of metastases is evidence that circulating tumor cells can infiltrate the primary tumor, contributing to its growth (Comen 2012). This "self-seeding" phenomenon has been observed in experimental models of breast, melanoma, and colon tumors (Kim, et al. 2009). These recent studies have provided alternative hypotheses to this stepwise model, influenced by the concepts of metastatic dormancy and cancer stem cells. Metastatic dormancy is defined as the ability of individual or small clusters of cancer cells to migrate to a distant site and then survive in a quiescent state for an extended period of time without growth (Aguirre-Ghiso 2007; Klein 2011; Nguyen, et al. 2009). The detection of tumor cells from circulation and bone marrow in patients thought cured from solid tumors based on imaging provides supporting clinical and experimental data. Well differentiated thyroid cancer and medullary thyroid cancer are two malignancy types where metastatic dormancy is a particularly common phenomenon (Ringel 2011) Many individuals after complete removal of their primary malignancy demonstrate persistent disease for years biochemically before metastatic disease or progression can be firmly identified on imaging. In these cases it is very likely that metastatic cells were present at diagnosis but were simply too small for detection since the primary tumor was completely removed and often times further treated with I-131 therapy. Additionally, individuals with defined distant metastases with thyroid cancer typically have prolonged disease stability, often without therapy, for years. In thyroid cancer tissues removed from patients with I-131 refractory residual disease, new gene mutations predicted to activate pathways beyond those of the initiating driver mutations have been described, consistent either with expansion of a subpopulation of cells from the primary tumor or the development of new mutations in the distant location (Ricarte-Filho, et al. 2009). Another important concept that may be involved in metastatic dormancy is the notion that cancer can be derived from putative cancer stem cells (CSC) or cells that express markers of pluripotent stem cells (Sampieri and Fodde 2012; Valent, et al. 2013). When these cells disseminate to distant sites, they may have the ability remain dormant in a quiescent state and have a slow proliferative rate until they are stimulated by their metastatic niche or by other secondary changes. Understanding the mechanisms of metastases dormancy and the subsequent escape to a progressive metastatic state will be crucial to guiding appropriate therapy for patients. These concepts and their potential impact on developing novel therapies for follicular cell-derived thyroid cancer are the focus of this review.

Metastatic Dormancy

Rupert Willis, a pathologist, first described dormant neoplastic cells when describing long-delayed metastatic tumors in a patient with no local recurrence after extirpation of the primary cancer in 1934 (Willis 1934). In 1954, the manuscript, "The Dormant Cancer Cell" appeared in the British Medical Journal as written by Hadfield (Hadfield 1954), where he describes a "temporary mitotic arrest" when noting latency periods in breast cancer and melanoma to be up to 20 and 32 years respectively. Clinical metastatic dormancy is defined when after removal of the primary cancer and further treatments have been finished, the time period of the disease-free course is longer than anticipated based on the expected growth rates of the metastatic disease. This phenomenon appears more common in certain types of cancers including breast, renal, prostate, melanoma, B-cell lymphoma, and well

differentiated thyroid cancer (Uhr and Pantel 2011). While clinical dormancy has been noted for many years, cellular dormancy has been appreciated through recent technological advancements via immunologic, cell isolation, and PCR-based assays which allow for the detection of small numbers of tumor cells in the circulating blood and bone marrow (Pantel, et al. 2008). Development of these sensitive biomarkers has confirmed that cancer cells can be detected in blood or tissues prior to radiographic or clinical confirmation. Cytokeratins are specifically expressed in epithelial cells; therefore, they have been used as antigens for antibody-mediated isolation of tumor cells from epithelial cancers such as breast, prostate, and colon in blood and bone marrow. Patients in whom circulating tumor cells (CTCs) are detected generally have a worse prognosis than those with no detectable CTCs even when they are detected many years after their primary cancer was treated. For example, it has been reported that more than 30% of breast cancer patients have detectable CTCs 7 – 22 years after mastectomy with no clinical evidence of disease (Meng, et al. 2004). Whether all of these cells have the ability for extravasation and outgrowth, or if their detection portends a poor prognosis in all patients in whom they are detected is uncertain as yet. Indeed, CTCs from patients with early breast cancer without overt metastases have been found to be genetically heterogeneous (Klein, et al. 2002). Additionally, CTCs from breast cancer have also been shown to have a low proliferative rate (Muller, et al. 2005). In fact, tumor cells found in bone marrow are largely non-proliferative (Sosa, et al. 2011). [Research showing the half-life of CTCs shortly after removal of a breast cancer was only 1 to 2 hours, suggests that there may be a secondary site acting as the source of these cells (Meng et al. 2004).] These cells have also been shown to have less global genomic instability than their corresponding primary lesions, suggesting dissemination may be an early event (Schardt, et al. 2005). Furthermore, in mouse models, non-transformed cells have also been shown to have the ability to survive for an extended period of time without growth in a distant sites (lung), but are later capable of malignant growth with the activation of oncogenes (Podsypanina, et al. 2008). Taken together, these data provide evidence that the process of metastatic dormancy may play a common event in certain cancers and may not require multiple genetic alterations as predicted in the sequential progression model.

How then do cancer cells remain dormant in metastatic microenvironments? The mechanisms are likely multiple including restraints in growth intrinsic to the surrounding microenvironment (e.g. physical modulus) and extracellular matrix, internal signaling pathways from oncogenes or metastasis suppressors, angiogenesis, and immunosurveillance.

The extracellular matrix (ECM) provides a physical and chemical structural support for cellular and tissue structure. It serves as a physical scaffold and provides substrates for cell adhesion, motility, and signaling. It has been argued that the ECM is as important as soluble signals in determining cellular differentiation, proliferation, polarity, angiogenesis, and survival (Hynes 2009). ECM proteins are typically large and complex, comprised of multiple highly conserved domains. Growth factors are known to bind avidly to ECM proteoglycans, which can establish important gradients to drive growth or migration. Some growth factors like FGFs and TGFβ require proteoglycan association before binding to their respective receptors. Considering the importance of the ECM, it has been suggested that when metastatic cells are in a non-fertile environment, dormancy is induced by the ECM-cell interaction. Several lines of evidence suggest that lack of significant adhesion by the

tumor cell to the ECM leads to a dormant state (Barkan, et al. 2010). The growth of breast cancer cell lines in a three dimensional matrix designed to mimic ECM or that includes ECM proteins recapitulated their dormant or proliferative pattern for growth *in vivo* better than their growth patterns on flat culture dishes. Cancer cell spherule growth *in vitro* has correlated with metastatic capacity *in vivo* in thyroid cancer models as well (Todaro, et al. 2010). There is also evidence that cellular stress occurs when a solitary tumor cell does not properly adhere to the ECM; thereby initiating long term survival mechanisms.

Multiple ECM proteins have been shown to play important roles in dormancy and cell signaling. For example, periostin is a component of the ECM which is expressed by normal fibroblasts in mammary glands. In order for primary breast tumors to initiate colonization in the lung, tumor cells need to induce stromal periostin expression, and blocking its function prevents metastasis (Malanchi, et al. 2012). Periostin is induced by TGF-β3 which binds Wnt ligands. Periostin null mice develop normal mammary glands and primary tumors, but do not develop metastases, suggesting its vital importance in the metastatic niche. Tenascin-C (TNC) is another ECM protein that appears to play a role in the formation of the metastatic niche for breast cancer in the lung (O'Connell, et al. 2011; Oskarsson and Massague 2012). TNC has been shown to increase Wnt and Notch signaling in the cancer cell. Like periostin, TNC null mice have decreased metastatic disease without affecting primary tumor growth.

The ECM also provides physical barriers which affect migration and invasion. Cancer cell migration is generally inhibited by the stiffness of the ECM, and the proteolysis of the adhesions between the cell and ECM is necessary for invasion to occur (Zaman, et al. 2006). Matrix stiffness also affects intracellular mechanical properties which is in part regulated through β1 integrins (Baker, et al. 2009). In thyroid cancer, multiple component proteins in this pathway are functional regulators of increased invasion or migration including urokinase plasminogen activator, Src kinase (SRC), focal adhesion kinase (FAK), and pre activated kinase (PAK) (McCarty, et al. 2010; Nowicki, et al. 2010; Schweppe, et al. 2009; Vasko, et al. 2007). A better understanding of the interactions of these different pathways with the ECM, may provide therapeutic targets for the metastatic microenvironment of thyroid cancer.

In addition to physical barriers to proliferation and growth, the cancer cells themselves may also maintain metastatic dormancy through expression of proteins encoded by metastatic suppressor genes (MSG). These are defined by their ability to inhibit metastases *in vivo* but not function as tumor suppressor genes (Horak, et al. 2008). The mechanisms by which they inhibit metastases are varied. The first MSG to be identified was Nm23-H1 in a melanoma cell line (Steeg, et al. 1988). Nm23-H1 has been shown to be a multifunctional enzyme and influence a variety of steps during the process of metastasis including invasion, survival, and colonization (Marino, et al. 2012). Several groups have investigated the role of Nm23-H1 in thyroid cancer and found it to be reduced in metastatic nodes and tissues (Arai, et al. 1993; Arai, et al. 1995). Others have found an association of reduced levels in the primary tumor with a poor prognosis in follicular but not papillary thyroid carcinoma (Zafon, et al. 2001). Lysophosphatidic acid receptor 1 (LPA1) is a G-protein-coupled receptor whose expression is inversely related to Nm23-H1. Inhibiting LPA1 has been shown to decrease metastases

but not the primary tumor in a mouse model suggesting manipulation of MSGs may have a therapeutic role (Marshall, et al. 2012). KiSS1 is another MSG which has been associated with dormancy of several cancer types in lung tissue. KiSS-1 encodes a family of secreted proteins known as Kisspeptins which also have been shown to be ligands for G-protein coupled receptor (GPR) 54 (Ohtaki, et al. 2001). GPR54 activation is coupled to $G_{q/11}$ and intracellular calcium signaling, but the role GPR54 in mediating the effect of Kisspeptins to maintain metastatic dormancy is not certain (Nash, et al. 2007; Stathatos, et al. 2005). Nonetheless, activation of GPR54 has been demonstrated to decrease migration and adhesion to ECM proteins by inhibit calcineurin signaling via enhanced expression of regulator or calcineurin 1-4 (RCAN1-4) (Espinosa, et al. 2009). Interestingly, the RCAN1 gene, which encodes all RCAN 1 family members, is also known as Down's syndrome candidate region 1 gene. Individuals with Down's syndrome have a reduced incidence of most solid tumors (Hasle, et al. 2000) and RCAN1 trisomy has been reported to be in part responsible for suppression of tumor growth and invasion in a mouse model of Down's syndrome (Baek, et al. 2009). This host effect likely is related to RCAN1-mediated inhibition of VEGF receptor signaling. Thus, RCAN1 may regulate metastatic progression through effects on both cancer cells and on the host-tumor interface. KAI1 is another metastasis suppressor which has been shown to cause senescence when a migrating tumor cell interacts with the Duffy antigen receptor on endothelial cells(Iiizumi, et al. 2007). Breast Cancer Metastasis Suppressor 1 can sensitize cells to apoptosis potentially through suppression of osteopontin (Wu, et al. 2012b). Thus, it seems likely that individual or groups of MSGs may function normally to restrain metastasis and growth of cancer cells through a variety of mechanisms. Loss of expression or function of these proteins may be mechanistically important in the escape of cancers from metastatic dormancy.

As previously mentioned, proliferating cells depend on the availability of substrates. For over a hundred years, it has been recognized that cancers are characterized by increased number and size of blood vessels (Ferrara 2002). In 1971 inhibition of tumor angiogenesis with subsequent loss of substrate delivery was proposed to be a potential treatment for cancer (Folkman 1971). Moreover, it was later suggested that lack of angiogenesis might be a possible factor supporting cancer dormancy using thyroid cancer as an example (Folkman and Kalluri 2004). Because of their rapid proliferation and abnormal vasculature, aggressive solid tumors possess regions where nutrients and oxygen are limiting. Oxygen availability is one of the primary regulators of new vessel formation. Hypoxia induces a transcriptional response mostly through increased hypoxia inducible factors (HIFs) to produce multiple angiogenic factors, including vascular endothelial growth factor (VEGF). VEGF induces quiescent endothelial cells to detach from their parent vessel and migrate into the neighboring stroma leading to angiogenesis (Krock, et al. 2011). VEGF plays a critical role in this process in many cancers including papillary thyroid cancer, where higher expression levels correlate with metastatic disease and decreased disease-free survival (Klein, et al. 2001; Lennard, et al. 2001). The ubiquitin E3 $SCF^{\beta\text{-}TRCP}$ ligase has been shown to suppress angiogenesis through destruction of VEGF receptor 2, and shown to be inversely correlated with angiogenesis in PTC (Shaik, et al. 2012). Other factors such as ECM degradation, recruitment of endothelial progenitor cells and smooth muscle are all required for the development and maintenance of blood vessels needed for the metastatic tumor mass to

proliferate. Recently the heat shock protein, HSP27, has been implicated in tumor dormancy in breast cancer through its regulation of VEGF and fibroblast growth factor (Straume, et al. 2012).

Inhibition of angiogenesis appears to have a central role in the activity of multikinase inhibitors in that have clinical activity in patients with progressive metastatic thyroid cancer. Over the past decade, a number of compounds have been designed to inhibit molecular pathways that are drivers of thyroid tumorigenesis and growth. Several small molecule inhibitors targeting important oncogenes including RET, BRAF, and Ras that commonly drive thyroid cancer development have gone to clinical trial (Haugen and Sherman 2013). Partial response rates ~30% have been seen, and prolonged but non-durable stabilization is even more common, consistent with a restoration of metastatic dormancy for a period of time. Many of these drugs inhibit a number of kinase targets and it is not clear if oncogene inhibition is responsible for their anti-progression effects. Of interest is that all of the active compounds thus far reported inhibit VEGF receptors (Sherman 2009). Thus, it has been postulated that inhibition of VEGF signaling is in part responsible for the stabilization and partial responses noted in clinical trials. As noted above however, the responses are transient and there are concerns that interrupting VEGF blockade may induce rapid tumor regrowth after initial response possibly related to the persistent basement membrane scaffolding from the prior vessels or other mechanisms (Ebos and Pili 2012; Kubota 2012). In fact sunitinib, a compound with activity against metastatic thyroid cancer, when given short-term has been reported to accelerate metastatic tumor growth, but not orthotopic “primary” tumors of breast and melanoma cancer cells in mice (Ebos, et al. 2009). While BRAF specific inhibitors have shown promise in melanoma cell lines and clinical trials in metastatic melanoma patients, thyroid cancer cell lines with BRAF mutations are comparatively resistant to BRAF inhibitors. Recent work suggests that one mechanism of secondary resistance in thyroid cancer cells is through HER3 transcription (Montero-Conde, et al. 2013). Despite this, a phase I trial of Vemurafenib (a selective RAF inhibitor) demonstrates a possible response in metastatic papillary thyroid cancer, but is only in three patients (Kim, et al. 2013). Ongoing studies will help to determine their therapeutic utilities. The mechanisms of resistance that develop in thyroid cancer patients treated with these compounds is an area of active study but may hold the keys to understanding the mechanisms that are sufficient to maintain metastases in a dormant state.

The immune system also plays a crucial role in regulating tumor growth in both the thyroid and metastatic environments. Immune cells can both inhibit and exacerbate metastatic progression. Since the concept of immunosurveillance of cancer was first proposed in the 1950's, the role of the immune system as an inhibitor of cancer growth and metastatic spread has been generally accepted, but the nature of this interaction appears to be remarkably complex. A recent model of the relationship between the immune system and cancer includes three stages through which tumors may progress: elimination, equilibrium, and escape (Oleinika, et al. 2013; Schreiber, et al. 2011), which lends itself well to a potential role for the immune system in maintenance of metastatic dormancy. In the “elimination” phase, innate and adaptive immunity attack developing tumors before they become clinically apparent. If a cancer survives the elimination phase, it enters the “equilibrium” or dormant phase, which is primarily an adaptive immune response. Finally, the tumor “escapes” and

further proliferation occurs. In experimental models, tumors can be eradicated through an adaptive immunity mechanism, which is largely T cell mediated. The primary T cells involved are the CD8+ cytotoxic T lymphocytes (CTL) which actively kill cells when tumor-associated or tumor specific antigens are recognized. They have the ability to keep B cell lymphomas and other tumors dormant (Farrar, et al. 1999). Evidence for the equilibrium stage comes from mouse models. In one model, after immunocompetent mice were treated with a low-dose carcinogen, no apparent tumors were noted for an extended period of time; but when the immune system was compromised after depletion of T cells and IFN-γ, sarcomas became apparent (Koebel, et al. 2007). In another mouse model, micrometastatic melanoma lesions demonstrated dormancy at least in part through CD8+ T cells (Eyles, et al. 2010).

Some tumors over time become less immunogenic and evade immunosurveillance, possibly through a mechanism of immunoediting, where their immunologic profile changes over time and they can enter the “escape” phase (Schreiber et al. 2011). This can occur through a variety of intrinsic and extrinsic mechanisms, such as selective loss of the tumor-specific antigen, lack of a co-stimulator expression, downregulation of MHC expression, or through an inhibitor of T cell function, such as CTLA-4 or PD-1. The role of the CD4+ T lymphocytes (helper T cells) is less well defined in the cancer immune response, but they comprise at least 50% of all circulating lymphocytes and clearly have an important role. Naive CD4+ T cells after exposure to cytokines, antigens, and other factors differentiate into at least four subtypes, Th1, Th2, TH17, or Treg (which are CD25+ Fox P3 regulatory T cells). There has been considerable interest in Treg function which are thought to be important in peripheral tolerance of cancer cells, and when shown to be increased in the tumor and peripheral blood of cancer patients are typically associated with a poor prognosis (Facciabene, et al. 2012). Recently this has been shown to be true for papillary thyroid cancer (French, et al. 2010). Treg frequency has been reported to be increased in involved papillary thyroid cancer lymph nodes, especially patients with recurrent disease(French, et al. 2012), suggesting a possible role for T cell exhaustion in thyroid cancer progression. Decreased survival in thyroid cancer patients has also been associated with tumor associated macrophage (TAM) density in primary tumors (Ryder, et al. 2008). TAMs were also associated with tumor grade and invasion and are known to secrete a large variety of chemokines and growth factors which can exert a paracrine effect on cancer cells. Inhibition of TAM recruitment using inhibitors of Colony Stimulating Factor 1 (CSF-1) signaling reduces tumor progression in mice with thyroid-specific expression of BRAF V600E (Ryder, et al. 2013), consistent with an important facilitating role for TAMs in thyroid cancer progression. There has long been an association with chronic inflammation and cancer, such as in Barrett's esophagus, pancreatitis, chronic skin wounds and autoimmune thyroid disease (Guarino, et al. 2010). Supporting this idea, are the known increases in angiogenesis and changes in the ECM associated with inflammation. The association between autoimmunity and the development of PTC has been described, although it is controversial if Hashimoto's thyroiditis is clearly associated with a higher incidence of PTC(Jankovic, et al. 2013). PTCs that arise in patients with underlying Hashimoto's thyroiditis have been reported to have a better prognosis than in patients without this condition (Lee, et al. 2013). PTCs also have been shown to induce lymphocytic infiltration

with populations of T cells that may correlate with likelihood of progression as noted above. It is of interest that when PTC is associated with thyroiditis, there is a potential link to RET/PTC1 oncoprotein expression while BRAF mutations are more likely when no thyroiditis is present (Muzza, et al. 2010). Normal human thyrocytes, when exogenously expressing RET/PTC1, increase expression of a multiple genes associated with inflammation including cytokines, metalloproteases, urokinase-type plasminogen activator, and an adhesion molecule (Borrello, et al. 2005). However similar pathways leading to immune response and also expression of proteins that degrade ECMs also have been shown in PTCs and in cell lines with BRAF V600E mutations *in vitro* and *in vivo* (Nucera, et al. 2010).

As the critical role of the immune system in cancer suppression and progression has been more clearly defined, Hanahan and Weinberg added immune evasion to the six hallmarks of cancer (Hanahan and Weinberg 2011). Orchestration of the immune system through immunotherapy (such as IL-2), vaccinations, or adoptive cell transfer (transfer of autologous T cells with antitumor activity) has already shown promise and may be able to exploit the system to achieve long term equilibrium or extinction (Rosenberg 2012). Whether or not this approach is appropriate for patients with progressive thyroid cancer is not certain.

Primary Dormancy or Latency

While the previous discussion relates to dormancy of the metastatic cells, the idea of primary tumor dormancy (also termed latency) is also an important component of thyroid cancer biology. In autopsy studies where select tissues are rigorously examined, there have been a high number of breast and prostate cancers noted suggesting a dormancy of the primary tumor (Nielsen, et al. 1987; Sakr, et al. 1995). This has also been demonstrated for thyroid cancer (Harach, et al. 1985). From 101 consecutive autopsies in Finland the prevalence of occult papillary carcinoma was 35%, which is much higher than the prevalence of clinically apparent papillary thyroid carcinoma in similar aged adults (Black and Welch 1993). Clinically, it is common to see patients with long-standing thyroid nodules or even cervical lymph nodes from thyroid cancer with no clinical progression. It is interesting that primary and metastatic dormancy have been seen in similar tumor types and may suggest and important intrinsic component to the particular tumor that may limit progression in several microenvironments. Thus, it may be possible to predict which thyroid cancers are likely to display metastatic dormancy or more rapid rates of progression.

Cancer Stem Cells

Stem cells are undifferentiated cells possessing the ability for indefinite self-renewal and also pluripotency (Davies, et al. 2011). Stem cells can be categorized into embryonic stem cells (ES) which are derived from the inner cell mass of the blastocyst and are pluripotent, and adult stem cells, which are typically found in differentiated tissue and considered multipotent. All stem cells are affected by their surrounding microenvironment or niche which contributes to their fate. When exposed to different milieu of hormones and growth factors, stem cells can differentiate into specific progenitor cell lineages which can further differentiate into functional cells.

Mouse ES cells were isolated from mouse embryos in 1981 (Evans and Kaufman 1981), and human ES cells were isolated in 1998 (Thomson, et al. 1998). The first mouse thyrocyte-like cell derivatives from ES cells was accomplished through exposure to thyroid-stimulating hormone (TSH), and could be shown to express a variety of genes associated with mature thyroid follicular cells, namely NIS, and PAX8 (Lin, et al. 2003). These cell populations were extremely heterogeneous and transient. Using a TSH receptor-based selection strategy, thyrocyte-like cell clusters could be grown on Matrigel and shown to take up iodine (Arufe, et al. 2006). Again, the population of thyroid-like cells was too low for further characterization and no thyroglobulin (Tg) expression was found (Thomas, et al. 2008). Thyroid follicular cells are derived from the endoderm germ layer which can be derived from mouse and human ES using Activin A. This allowed a TSH-independent induction of thyroid progenitors (Ma, et al. 2009). By using a transcription factor TTF-1 (Nkx2-1) GFP reporter knock-in ES cell line, stage specific inhibition of BMP and TGFβ was used, from which progenitor lines with lung and thyroid characteristics were obtained (Longmire, et al. 2012). Recently, mouse ES cells which have been engineered to transiently overexpress TTF-1 and PAX8, differentiate into thyroid-like follicular cells, which when treated with TSH, organize into three-dimensional follicles (Antonica, et al. 2012). When grafted into athyreotic mice, this tissue was able to rescue thyroid hormone deficiency. Further advances in the understanding of ES cell thyroid differentiation will provide insight into the mechanisms of thyroid development and possibly cancer.

Adult stem cells are purported to maintain normal homoeostasis of the daily turnover of cells and also provide a regenerative response to injury; and therefore can be either quiescent or cycling, depending on the need of the organ. Adult thyroid stem cells have long been posited (Dumont, et al. 1992), but human thyroid follicular cells have are estimated to have a turnover rate of ~8.5 years which translates into about five divisions during the course of an adult lifetime, making their identification and isolation challenging (Coclet, et al. 1989). Factors regulating the total numbers of cells and cell growth are not completely known. When human multinodular goiter tissue was transplanted into nude mice, a small portion of the cell population (up to 7%) proliferated despite a suppressed TSH (Peter, et al. 1985). The presence of a potential adult thyroid stem cell in solid cell nests in normal human thyroid was suggested by the expression of p63 (a p53 homologue) and all cytokeratins but cytokeratin 20 (Reis-Filho, et al. 2003). Oct-4 is a stem cell marker and GATA-4 and HNK4alpha are early endodermal markers which have been seen in human thyroid tissue and cultured cells from goiters; again suggesting the existence of adult stem cells (Thomas, et al. 2006). A side population of potential adult thyroid stem cells was separated by fluorescence-activated cell sorting (FACS) by ABCG2 transporter expression (a marker of “stemness”) of thyrocytes cultured from human goiters (Lan, et al. 2007). Growth stimulation of these cells resulted in a high proliferative rate, but under TSH stimulation, expression of PAX8, TSHR, NIS, and Tg occur and there is loss of stem cell markers. Fierabracci, culturing fresh surgical thyroid specimens with EGF and bFGF was able to obtain self-replicating clonally derived spheroids many of which did not express Tg, TPO, TSH-R, and NIS (Fierabracci, et al. 2008). When these spheroids were grown on collagen gels with ‘differentiation medium’ about half of them would form follicules and produce T4.

When spheroids were co-cultured with a neuroblastoma cell line, they expressed the neuronal marker beta-tubulin III suggesting the cells are multipotent.

Over the past two decades, the concept of cancer stem cells (CSC) which have the ability for self-renewal and can differentiate into multi-lineages has emerged. Although controversial, it has been proposed that CSCs represent the foundation of cancer initiation and metastasis. The regulatory switches that maintain homeostasis by balancing between quiescence and division found in the normal adult stem cell are lost in the CSCs. Therefore, in this model, instead of the traditional multiple hit process to change a differentiated thyroid follicular cell into a cancer cell, a thyroid stem cell undergoes changes resulting in a balance shift toward proliferation without differentiation (Lin 2011). In the multistep theory, anaplastic thyroid cancer are postulated to be derived from preexisting more slowly growing differentiated thyroid cancer through the gain of additional mutation(s), (such as loss of TP53) or further loss of tumor suppressors (Sarlis 2000). This model is supported by the tendency of anaplastic thyroid cancers to be identified either within or coexistent with differentiated thyroid cancer elements. Evidence to support a separate pathway of origin between anaplastic and well-differentiated thyroid cancers are the existence of specific mutations typically found only in differentiated thyroid cancer, i.e. RET/PTC and PAX8-PPARγ1 rearrangements are only reported in papillary and follicular carcinomas respectively, and are rarely seen in anaplastic carcinomas (Dwight, et al. 2003; Nikiforova, et al. 2002; Tallini, et al. 1998). Moreover, the common PTC mutation BRAF V600E is identified in only about a quarter of ATCs (Smallridge, et al. 2009). Finally, in some cases, anaplastic cancers appear to arise quickly and without a differentiated component. To explain these situations, an alternative “stem cell” hypothesis for thyroid cancer development has been proposed in which the different types of thyroid cancer are derived from unique initiating cell types (Takano 2007). Namely, anaplastic cancer is derived from adult thyroid stem cells, and papillary and follicular are derived from progenitor lineages of the thyroid stem cell, thyroblasts and prothyrocytes, respectively

Isolated CSCs , or cancer cells with stem cell marker expression, represent a small population within the large mass of the primary tumor that have the ability to self-renew as well as generate progeny that are more differentiated (Baccelli and Trumpp 2012). CSC have been identified and propagated from both hematopoietic malignancies and a wide variety of solid tumors (Sampieri and Fodde 2012). Many investigators have used aldehyde dehydrogenase (ALDH), an enzyme expressed in stem cells and found to influence their regulation, to identify and isolate possible CSCs in a variety of primary tumors (Chute, et al. 2006). ALDH represent multiple families and over 19 genes in humans (Muzio, et al. 2012) and are able to convert a substrate into a fluorescent product allowing separation during FACS. High ALDH has helped identify potential CSCs in breast (Croker, et al. 2009; Ginestier, et al. 2007), lung (Jiang, et al. 2009), and squamous cell head and neck cancer (Chen, et al. 2009), and to be a marker for metastatic ability in immunosuppressed mice (Charafe-Jauffret, et al. 2010; Charafe-Jauffret, et al. 2009). High ALDH expression has been used to identify a subpopulation of stem-like cells from 26 primary thyroid cancers (Todaro et al. 2010). The identified cells were a small population of the total tumor cells: 2% of papillary, 1.2% of follicular, and 3.5% of undifferentiated, and were able to expand

indefinitely as tumor spheres *in vitro*, while the remaining other cells only lasted 2 weeks in culture. These spheres also generally expressed stem cell associated CD44, Oct3/4 and Nanog. Upon heterotopic injection, cells with high levels of ALDH were able to grow starting with as few as 5×10^3 cells when injected into immunocompromised mice. Serial transplantation was successful from the tumors, which is one of the established characteristics of stem cells. When only 100 cells were injected into the thyroid of immunocompromised mice, tumors would form within 4 weeks. CSC populations derived from undifferentiated thyroid cancers when injected orthopically caused local compromise of the trachea and esophagus and form cervical lymph node and lung metastases. Knockdown of cMet and AKT using shRNA in the undifferentiated thyroid cancer-derived cells decreased tumor growth and metastasis formation. These findings may lead to an important preclinical tool improved understanding of the metastatic process and an avenue to evaluate potential therapies (Lloyd, et al. 2013).

Although there is speculation that the CSCs found in primary tumors may be the cell of origin of distant metastases, no clear causal relationship has been shown. Recent investigations have revealed a large degree of metastatic genetic heterogeneity in several tumor types (Campbell, et al. 2010). In primary and metastatic renal cell carcinoma, over 60% of somatic mutations were not carried across all tumor regions (Gerlinger, et al. 2012). In medulloblastoma, metastatic lesions were quite divergent from matched primary tumors (Wu, et al. 2012a). This marked genetic diversity may make targeted cancer therapy more difficult, especially when treating metastatic disease. This heterogeneity may also apply to the CSC population. For example, it has been shown in pancreatic cancer that the invasive fronts contain a distinct subpopulation of CSCs characterized by CXCR4 expression which was shown to affect the migration and be a critical factor supporting metastatic progression (Hermann, et al. 2007). This and other work has led to a dynamic view of CSC, where distinct CSC may exist or evolve within a tumor, where only a subset has the ability to metastasize.

Just as normal adult stem cells may cycle between a quiescent and actively proliferating state based on the need of an organ a similarly regulated process has been proposed for CSCs that might be controlled in part by the tumor microenvironment (Roesch, et al. 2010) (Kusumbe and Bapat 2009). The chemotherapy resistant nature of CSC contributes to the idea that metastatic CSC may persist in a dormant state, only later to change into a proliferative state to be detected as clinical metastases.

Summary and Clinical Implications

Distant metastasis is relatively uncommon in thyroid cancer and when it occurs, long-term stable disease is the typical clinical course. Unfortunately, when thyroid cancers progress, either at the time of diagnosis or after a period of dormancy, the best treatment responses are non-durable partial responses or stable disease, consistent with the conversion of the aggressive tumor back to a dormant or at least less proliferative balance. Thus, it is particularly crucial to better define the processes by which cancers metastasize and/or gain or lose dormancy in the primary and metastatic settings. Several models have been proposed (**Figure 1**) and it is not clear if all or one of these mechanisms occur within individual

thyroid cancers. Nonetheless, it is likely that the processes that regulate the development and progression of metastases likely include factors intrinsic to the cancer cell as well as factors in the host that may vary between different metastatic locations. Given the fairly rapid development of resistance to many current kinase inhibitors, we speculate that future therapies of metastatic follicular-derived thyroid cancer will still be based on the initial current classic treatment (surgery, radioactive iodine, and hormonal) followed by multiple, specifically-targeted therapies directed against the individualized driving mutations for their growth and mechanisms of resistance. This may include multimodality approaches. If the factors or mechanisms responsible for dormancy can be defined; it is possible they could be exploited to improve outcomes for patients with progressive metastatic disease by developing biomarkers to better predict the anticipate course for particular patents or by designing therapies directed against the causes of primary or secondary treatment resistance.

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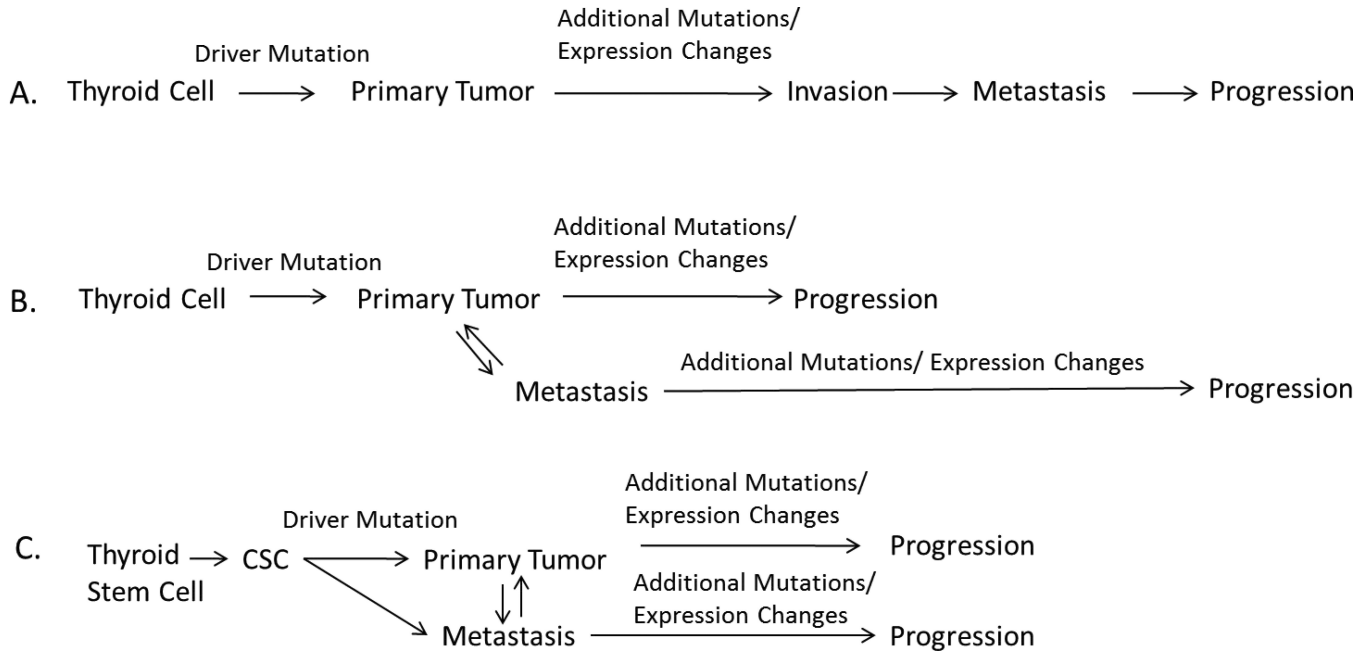

Figure 1. Models of Cancer Progression

Panel A depicts the classical model of cancer metastases in which metastasis is a late-stage event. An initial driver mutation results in the primary tumor cell, which through a series of genetic and epigenetic events, develops an invasive (malignant) phenotype eventually leading to metastasis and clinical progression of the disease. **Panel B** depicts a tumor dormancy model whereby cancer cells metastasize earlier in the life of the tumor and gain additional mutations in both the primary site leading to clinical and pathologic progression in both locations. In this model, disease progression may be influenced by communication between the primary and metastatic sites until the primary tumor is removed. **Panel C** depicts the cancer stem cell (CSC) hypothesis where progenitor cancer cells that develop early in tumorigenesis (either with or without the driver mutation) metastasize and lay dormant until additional alterations occur. In all cases the tumor environment plays a role in regulating progression in the metastatic site as described in detail in the text.