Fetal Cell Carcinogenesis of the Thyroid: A Hypothesis for Better Understanding of Gene Expression Profile and Genomic Alternation in Thyroid Carcinoma

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Abstract. Since the 1980s, cancer cells have been considered to be generated from well-differentiated benign cells by transformation caused by accumulating damage in their genomes. However, recent progress in gene expression analysis in thyroid malignancies has raised the possibility of another model of thyroid carcinogenesis. We propose a novel hypothesis of thyroid carcinogenesis, the fetal cell carcinogenesis hypothesis, in which cancer cells are derived from the remnants of fetal thyroid cells, instead of from normal thyroid follicular cells. This hypothesis explains well the clinical and biological features and recent molecular evidence of thyroid carcinoma. It suggests the importance of clarifying the molecular mechanism of thyroid development and the identification of fetal thyroid cells such as thyroid stem cells (TSCs), since such data will lead to a better understanding of thyroid carcinogenesis and thyroid regeneration.

Key words: Thyroid follicular carcinoma, Anaplastic transformation, Cancer stem cell, Multi-step carcinogenesis

The “fetal cell carcinogenesis” hypothesis — A new trend in thyroid cancer research

Thyroid tumors are relatively common, especially in women. Thyroid carcinomas are usually slow in growth, and fatal cases are rare, except for anaplastic carcinomas which show a relentless and deadly clinical course with rapid progression and dissemination [1]. Since the 1980s, cancer cells, including those of the thyroid, have been considered to be derived from well-differentiated normal cells, such as thyroid follicular cells (thyrocytes), via multiple incidents of damage to their genome, especially in oncogenes or anti-oncogenes that accelerate proliferation or foster malignant phenotypes, such as the ability to invade the surrounding tissue or metastasize to distant organs [2]. According to this hypothesis, in multi-step carcinogenesis, follicular carcinomas are generated from follicular adenomas, while papillary carcinomas are derived from some unknown precursor cells generated by normal thyrocytes. Anaplastic carcinomas are generated by both follicular and papillary carcinomas by genomic changes, such as mutations in TP53 [3] (Fig. 1).

Some of the recent molecular findings in thyroid carcinoma have raised questions regarding this model of thyroid carcinogenesis. For example, mutations in TP53, which are most often observed in anaplastic carcinomas, have been recognized to be closely related to the aggressive features of these carcinomas. However, mutations in TP53 are not necessarily responsible for the aggressive features of anaplastic carcinomas, since
no mutation in TP53 have been observed in a considerable percentage of anaplastic carcinomas, and these mutations are also observed in other types of tumors, even follicular adenomas [4, 5]. Tallini et al. examined the rearrangement of the RET gene in both anaplastic carcinomas and differentiated carcinomas, and found that rearrangement of the RET gene is limited in papillary carcinomas and never observed in anaplastic carcinomas [6]. Furthermore, recent studies have shown that the PAX8-PPARγ1 rearranged gene is detected only in follicular tumors, not in anaplastic carcinomas [7–9].

In 2000, we proposed a novel hypothesis of thyroid carcinogenesis, the “fetal cell carcinogenesis” hypothesis, in which cancer cells are derived from the remnants of fetal thyroid cells instead of thyrocytes [10]. However, this idea did not attract much attention until an article by Rey et al. was published, in which they suggested striking parallels between stem cells and cancer cells [11]. After Singh et al. proved that brain tumors actually arise from stem cells (cancer stem cells), a considerable number of researchers have come to believe that cancer cells are derived from immature progenitor or stem cells, and not from well-differentiated cells [12–15]. In this review, we summarized our hypothesis of fetal cell carcinogenesis and its possible impact on the diagnosis of and therapy for thyroid carcinoma.

**From what is thyroid carcinoma derived?**

The most interesting fact in the recent studies on gene expression in thyroid tumors is that the expression of a fetal protein, oncofetal fibronectin (onfFN), is strictly limited to two carcinomas, papillary and anaplastic carcinomas [16–19]. Morphologically, these two carcinomas usually do not form follicles. Follicular tumors and papillary carcinomas express thyroglobulin (Tg) mRNA but anaplastic carcinomas do not [20]. Considering these facts, thyroid tumors can be arranged in the order of follicular tumors (adenoma and carcinoma), papillary carcinoma and anaplastic carcinoma.

The above considerations are linked to the key question, “From what is thyroid carcinoma derived?” The fetal thyroid originates in the pharynx and gradually moves to the front of the neck as it slowly grows. This indicates that fetal thyroid cells have the ability to move through other cells, which is similar to the ability to induce invasion or metastasis, and that they grow slowly, which are the similar characteristics we observe in differentiated thyroid carcinomas. Furthermore, in the mouse thyroid, fetal thyroid cells express thyroglobulin before they form follicles [21]. Because onfFN mRNA is known to be expressed in a wide variety of fetal tissues, the fetal thyroid is likely to express onfFN mRNA [22]. These facts indicate that some fetal thyroid cells have characteristics quite similar to those of thyroid papillary carcinoma cells. It is thus more reasonable to assume that thyroid carcinomas are generated directly from the remnants of fetal thyroid cells, rather than from rarely proliferating thyrocytes by de-differentiation.

Two previous reports strongly support this idea. First, in the Chernobyl accident, radioactive iodine, which does not have the ability to induce thyroid cancer in adults, induced papillary carcinomas in young children and infants. Interestingly, although this happened in a mildly iodide-deficient area, in which follicular carcinomas usually make up a considerable percentage of thyroid malignancies, almost all of the radiation-induced tumors were papillary carcinomas [23]. These facts strongly suggest that thyroid carcinomas, especially papillary carcinomas, were derived from some unknown source which exists only in young children or babies but not in adults. Second, Jhiang et al. found that RET/PTC1 transgenic mice develop thyroid papillary carcinoma. Interestingly, transgenic mice expressing a high level of the RET/PTC1 protein developed both papillary carcinoma and congenital hypothyroidism due to their lack of normal thyroid follicular cells [24]. These authors attributed the observed hypothyroidism to the de-differentiating effect of RET/PTC1 protein by which all the differentiated thyrocytes were transformed into cancer cells. Theoretically, these results were predicted when fetal cell carcinogenesis were taken into consideration, since hypothyroidism due to the lack of normal thyroid follicular cells occur eventually in these mice if the transgene RET/PTC1 prevents the differentiation of fetal thyroid cells into thyrocytes. The cells they observed in these transgenic mice might not be thyroid papillary carcinoma cells but might be fetal thyroid cells.

**The fetal cell carcinogenesis hypothesis**

We proposed a new model of thyroid carcinogenesis,
which we termed the “fetal cell carcinogenesis” model. In multi-step carcinogenesis, thyroid carcinomas are generated from thyrocytes via multiple genomic changes that foster the development of their cancerous characteristics. In fetal cell carcinogenesis, on the other hand, thyroid carcinomas are derived from the remnants of fetal thyroid cells which, unlike normal thyroid follicular cells, have the ability to migrate to surrounding tissues (Fig. 2). During the normal course of development, these characteristics of fetal thyroid cells are shown only in limited situations, whereas once fetal cells are transformed into cancer cells, they are no longer kept under control.

This hypothesis has two evident advantages over conventional multi-step carcinogenesis. First, micro-papillary carcinomas, which grow quite slowly and are often observed in autopsies, show a distinct morphological difference from normal follicular cells [25]. It is hard to believe that these carcinoma cells obtain their cancerous characteristics via multi-step carcinogenesis, since they do not divide many times before they are recognized as carcinomas, and thus are not likely to have undergone a dramatic change. In fetal cell carcinogenesis, on the other hand, these cells are considered to be derived from thyroid fetal cells, which already possess the potentially cancerous characteristics. Thus, they already resemble cancerous cells before proliferation.

Second, in fetal cell carcinogenesis, genomic changes in cancer cells do not play a major role in the expression of cancerous characteristics, unless such changes, for example, RET/PTC, prevent the fetal cells from differentiation, whereas the expression of fetal protein mRNAs is considered to prove that these cells are remnants of fetal cells. Thus, fetal cell carcinogenesis, not multi-step carcinogenesis, explains why cancer cells simultaneously show consistency in gene expression profiles and variation in genomic changes [26, 27].

Close relationship between development and carcinogenesis in the thyroid

Gene expression profiles in thyroid carcinomas suggest at least three types of fetal thyroid cells as their origins [28]. Thyroblasts express both Tg and onfFN mRNA and do not form follicles. Prothyrocytes, which are more differentiated than thyroblasts, express Tg mRNA but not onfFN mRNA, and have the ability to form follicles. The gene expression profiles of anaplastic carcinomas suggest the existence of quite undifferentiated fetal cells as their origin. We might call them thyroid stem cells (TSCs), since stem cells are defined as rare cells that have the ability to perpetuate themselves through self-renewal and to generate mature cells of particular tissues through differentiation [11].

Fig. 3 summarizes the concept of fetal cell carcinogenesis. Follicular tumors, papillary carcinomas, and anaplastic carcinomas are derived from the remnants of prothyrocytes, thyroblasts, and TSCs, respectively. TSCs, thyroblasts, and some prothyrocytes possess cancerous characteristics, and as they proliferate, they act as cancers. Any event that prevents fetal thyroid cells from differentiation can be a cause of cancer. It is suggested that RET/PTC and PAX8-PPARɣ rearrangements and mutations in BRAF contribute to such events [29]. Fetal cell carcinogenesis regards carcinogenesis as an abnormal development of fetal thyroid cells, but not de-differentiation of normal or benign thyroid follicular cells.

Fetal cell carcinogenesis and clinical features of thyroid carcinoma

Differentiated thyroid carcinomas often cause distant metastases and local invasion but they generally have a
good prognosis because they grow very slowly [30]. These features of thyroid carcinomas are quite unique and an adequate explanation of them has yet to be presented. In fetal cell carcinogenesis, cancer cells reflect the biological characteristics of their origins, and differentiated carcinomas are mainly derived from thyroblasts. We can easily understand the clinical features of differentiated thyroid carcinomas when we take the characteristics of the thyroblasts in the fetus into consideration. They have a high ability of migration and a low ability of growth, which are characteristics identical to those of differentiated thyroid carcinomas.

**Fetal cell carcinogenesis and thyroid follicular carcinoma**

One of the most difficult distinctions in thyroid pathology is the differentiation between benign follicular adenoma and carcinoma. The principal differentiating feature is capsular invasion. Even this, however, may not be definitive, because slight capsular penetration can also be observed in benign tumors. Furthermore, both types of tumors have varying degrees of cellular atypia, and extensive invasion into vascular spaces is not usually observed in minimally invasive carcinomas. Preoperative diagnosis of follicular carcinoma is even more difficult, since the feature that separates the benign tumors from malignant tumors is the presence of capsular or vascular invasion, which is not possible to determine cytologically [31]. To solve this problem, much effort has been made to find genes that are differentially expressed in follicular carcinomas and adenomas. However, these trials have not been successful due to the variation of gene expression in thyroid follicular carcinomas [32]. This is not surprising when fetal cell carcinogenesis is taken into account. As shown in Fig. 4, since follicular tumors are placed at the very end of differentiation, follicular tumors can be derived from various origins through various ways, for example, from TSCs, thyroblasts, or prethyrocytes, which may lead to the variation in gene expression, whereas anaplastic and papillary carcinomas are derived from TSCs and thyroblasts, respectively, almost always in direct ways.

How can we distinguish follicular carcinomas from adenomas? In fetal cell carcinogenesis, thyroid tumor cells are generated during the normal course of thyroid development. Fetal thyroid cells lose their cancerous characteristics as they differentiate into thyrocytes. Thus, we can speculate that follicular tumor cells derived from a place far from normal thyrocytes are more likely to be malignant, whereas those derived from a place close to normal thyrocytes are more likely to be benign. In addition, we can consider that follicular tumors with a gene expression profile similar to that of thyrocytes are more differentiated and more likely to be benign.

In light of these considerations, we searched for genes overexpressed in normal thyroid and follicular adenomas but not in follicular carcinomas, and determined trefoil factor 3 (TFF3) mRNA to be a candidate. As expected, a low expression level was observed in all cases of definite follicular carcinoma, including the widely invasive type and the minimally invasive type with evident distant metastases, which indicated that TFF3 mRNA is a useful marker for distinguishing malignant from benign follicular tumors [33].
Anaplastic transformation

In multi-step carcinogenesis, anaplastic carcinomas are considered to be generated from differentiated carcinomas by an accumulation of genomic changes (Fig. 6A). This was hypothesized because anaplastic carcinomas often arise from long-existing differentiated carcinomas, and co-existence of a differentiated carcinoma and an anaplastic carcinoma is often observed [34]. However, as we described previously, it is hard to explain some of the genetic events observed in anaplastic carcinomas with this hypothesis.

TSCs possess the ability to produce differentiated cells and the ability of self-renewal. When anaplastic carcinomas are derived from the remnant of TSCs, the co-existence of differentiated carcinomas and an anaplastic carcinoma is often observed [34]. However, as we described previously, it is hard to explain some of the genetic events observed in anaplastic carcinomas with this hypothesis.

From a therapeutic point of view, the above theory provides another aspect to be considered. Thyroid carcinomas usually have a favorable prognosis. However, some tumors, especially those in elderly patients, take a fatal course [30, 31]. The precise molecular mechanism of this difference is not clear. As described above, a remnant of TSCs is a risk factor for anaplastic trans-

**Fig. 4.** Developmental course of thyroid follicular tumors in fetal cell carcinogenesis. Anaplastic carcinoma and papillary carcinoma are generated from their direct origins (white arrows), where as follicular tumor can be generated from various origins through various ways (broken arrows).

**Fig. 5.** Generation of malignant follicular tumors and benign follicular tumors. Cells that stop differentiation close to thyrocytes are likely to be benign, whereas cells that stop differentiation far from thyrocytes are likely to be malignant.
formation. Further, it is easily imagined that tumors with a remnant of TSCs are likely to take an unfavorable clinical course, since even if a tumor is dissected surgically, it will grow back because of the cells’ unlimited ability of proliferation. In contrast, with tumors that do not contain TSCs, the tumors are likely to shrink after surgical dissection since they have limited proliferative potential (Fig. 7). It might be important to recognize TSCs as the target of therapy, since if a therapy effectively kills the TSCs, the tumors are rendered incapable of maintaining themselves or growing.

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References

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