The Basic Theory of Fetal Cell Carcinogenesis of the Thyroid

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Abstract

Thyroid cancer cells have long been believed to be generated by multi-step carcinogenesis, in which cancer cells are derived from well-differentiated thyrocytes via malignant transformation caused by multiple damages to their genome, until a new hypothesis, fetal cell carcinogenesis, pervaded. In fetal cell carcinogenesis, thyroid tumor cells are assumed to be derived from one of the three types of fetal thyroid cells, namely thyroid stem cells, thyroblasts and prothyrocytes, which exist in fetuses or young children and are the origin of anaplastic carcinoma, differentiated carcinoma and follicular adenoma, respectively, by proliferation without differentiation. Genomic alternations play an oncogenic role by preventing thyroid fetal cells from differentiating. Fetal cell carcinogenesis effectively explains recent molecular and clinical evidence regarding thyroid cancer, including the existence of thyroid cancer initiating cells. It also underscores the importance of identifying and analyzing fetal thyroid cells, including thyroid stem cells, and clarifying the molecular mechanism of organ development. Furthermore, fetal cell carcinogenesis introduces three important concepts: reverse approach, stem cell crisis, and mature and immature cancers.

Keywords: Thyroid cancer, progenitor, reverse approach, stem cell crisis, immature cancer

Is cancer really Godzilla?

Godzilla is a giant brutal monster in a movie. The majority of researchers regard cancer as an analogue of Godzilla. Godzilla was originally a small, innocent creature that was transformed by radiation of an atomic bomb. A cancer cell described in multi-step carcinogenesis looks exactly like this, as it is produced by the transformation of a normal cell that did not possess cancerous characteristics such as invasiveness or metastatic potential (1). In the thyroid, “a normal cell” is usually regarded to be a thyroid follicular cell (thyrocyte). Thyrocytes, which take an important role in thyroid hormone production, do not show the ability of invasion or metastasis, and they rarely proliferate. In the classical model of thyroid multi-step carcinogenesis, thyrocytes gradually transform into malignant cells during proliferation due to multiple damages of their genome. Once they start their transformation, the tissue continues to change from normal thyroid to follicular adenoma, differentiated carcinoma, poorly differentiated carcinoma, and finally, anaplastic carcinoma (2, 3).

Even though the above hypothesis has long been believed and cited in many textbooks on the thyroid, there is no direct evidence proving that thyrocytes actually transform into tumor cells. It is true that the co-existence of thyrocytes and thyroid tumor cells and that of differentiated carcinoma cells and anaplastic carcinoma cells are observed, but the evidence that well differentiated cells can actually transform into undifferentiated cells are scarce (4). Accumulation of multiple damages in thyroid cells has been regarded as the cause of tumor progression. However, recent molecular evidence in thyroid tumors does not agree with this hypothesis. For example, RET/PTC and PAX8/PPARγ1 rearrangements are observed in high prevalence in differentiated carcinomas, but they are not detected in anaplastic carcinomas (5, 6). In five major studies carried out in Japan targeting patients with similar ethnic and environmental backgrounds, BRAF mutations were found in 39.7 % (378 out of 951) of papillary carcinomas, but only in 18.1 % (6 out of 33) of anaplastic carcinomas, even though papillary carcinoma occupies about 90% of differentiated thyroid carcinoma in Japan (7-11). These data present serious doubt about the widely believed concept of anaplastic transformation, in which differentiated tumor cells are transformed into anaplastic carcinoma cells. Furthermore, the starting point of multi-step carcinogenesis is thyrocytes which rarely proliferate (12). Therefore, it is hard to believe that a thyrocyte undergoing only a few divisions happens to acquire so-called cancerous characteristics which consist of many biological features.

The above consideration brings us to the idea that, at least in the thyroid, a cancer cell might not be like Godzilla, which transformed from something benign. Then we have to face to a fundamental question, “What does a cancer cell derive from?”

Some important evidence to speculate the origin of thyroid carcinoma

There is some important evidence to focus on when we try to figure out the above question. Radiation increases the risk of thyroid carcinoma in young children but not in adults. Adult patients with Graves’ disease undergoing radiiodine therapy (1³¹I) do not suffer from radiation-induced thyroid cancer (13). After the Chernobyl nuclear plant accident, radiation-induced thyroid cancer was observed mainly in children under age 5 but not in adults (14). These results have been explained to be due to the high proliferation rate of thyrocytes in the young children. However, the proliferation rate of thyrocytes remains constant from birth to age 10 and thyrocytes keep proliferating slowly, even in adults (15). Thus, an evident discrepancy is observed between the proliferation rate of thyrocytes and cancer risk.

Experiments using transgenic mice have proved that oncogenes such as RET/PTC1 or mutated BRAF in the thyroid can induce thyroid cancer only when they are expressed under the control of thyroglobulin promoter during the fetal period (16-18). Interestingly, RET/PTC1 or mutated BFAF transgenic mice...
develop not only papillary carcinoma but also congenital hypothyroidism due to the lack of thyrocytes. These results indicate that thyrocytes disappear when an oncogene is expressed in all cells expressing thyroglobulin, and suggest that both thyrocytes and thyroid cancer cells are derived from the same original cells which exist only in the fetal period. The results also suggest that introduction of an oncogene forces the original cells to turn into cancer cells but not into thyrocytes. Charles et al. found that the expression of mutated BRAF induces papillary carcinomas and hypothyroidism due to the lack of thyrocytes as described in some previous studies and that pharmacologic inhibition of the mutated BRAF signal leads to restoration of thyrocytes and hormone production, suggesting that papillary carcinoma cells differentiate into thyrocytes (19). These results have suggested that papillary carcinoma cells are produced with a single hit in their genome, and that papillary carcinoma cells observed in this study could turn into thyrocytes by inhibiting the activity of an oncogene.

From these results, we could obtain some important information on thyroid carcinogenesis: 1) thyrocytes and cancer cells are derived from the same original cells which exist only in fetus or young children but not in adults; 2) an oncogene produces carcinoma cells by forcing the original cells, which otherwise change into thyrocytes, to turn into cancer cells; and 3) carcinogenesis occurs in a single step rather than in multiple steps, and is reversible.

**Human fetal thyroid cells**

>From above observations, embryogenesis and development of fetal thyroid may contain a new insight to the unsolved question in thyroid carcinogenesis. In a previous study, at least three types of fetal thyroid cells were observed (20). The fetal thyroid is first recognized as a small number of undifferentiated cells at the lingual radix. They are determined to be the origin of thyrocytes since they express thyroid transcription factor-1 (TTF-1, NKX2-1), but they do not express thyroglobulin (TG). Without evident proliferation, these cells move down from the lingual radix toward the front neck. Even though it is not clear whether these cells show pluripotency, they are called thyroid stem cells (TSCs) since thyroid development starts from these cells. TSCs soon become cells expressing TG, called thyroblasts. Thyroblasts do not form thyroid follicles and keep moving to the front neck. The thyroid volume increases gradually due to the proliferation of thyroblasts. Next, fetal thyroid cells start forming follicles, but they do not produce thyroid hormone. Fetal thyroid cells during this period are called prothyrocytes. Finally, after settling in the front neck, fetal thyroid cells turn into thyrocytes, and then start producing thyroid hormone.

Each of these three fetal thyroid cells has its corresponding analogue in thyroid tumors. TSCs are similar to anaplastic carcinoma cells since they are poorly differentiated. Thyroblasts show similarities to differentiated thyroid carcinoma cells in the expression of TG in addition to their high mobility and low growth rate. Prothyrocytes are similar to follicular tumor cells since they form follicles but most of them do not produce thyroid hormone. Oncofetal fibronectin (onfFN), a fetal protein, is a splicing variant of normal fibronectin, and expressed in various fetal tissues at the early stage of thyroid development (21). Thus, it is likely that the fetal thyroid cells at the early developmental stage such as TSCs and thyroblasts express the onfFN. Expression of onfFN is observed in anaplastic carcinoma and papillary carcinomas in a restricted manner and its weak expression is also observed in some follicular carcinomas, suggesting the close relationship between early fetal thyroid cells and thyroid carcinomas (22, 23).

**The basic concept of fetal cell carcinogenesis**

In the classical model of multi-step carcinogenesis, cancer cells are generated through an adenoma-carcinoma sequence as described in Fig.1A. Tumor cells have been described to be derived from “a normal cell”. In thyroid, there is no definite description regarding what a normal cell is in some textbooks and in others a normal cell is regarded as a thyrocyte (2, 3). Some modifications are needed in this figure to describe carcinogenesis more correctly. All cells in human, including those in the thyroid, derive from the stem cells. Thus, the very starting point of this story is not “a normal cell” but a stem cell. Thus, in the figure showing adenoma-carcinoma sequence, the process that a stem cell differentiates into a normal cell should be added before a normal cell de-differentiates into tumor cells. In such a figure shown in Fig. 1B, the mechanism of carcinogenesis looks quite complicated since malignant tumor cells are generated after a long trip of repeated differentiation and de-differentiation starting from a stem cell.

![Figure 1: The basic concept of fetal cell carcinogenesis. 1A: the adenoma-carcinoma sequence; 1B: the adenoma-carcinoma sequence with normal fetal cell development; and 1C: the relationship between normal fetal cell development and fetal cell carcinogenesis.](image)

However, when we take a close look at this figure, we can find a shortcut. Developing fetal cells possess the ability of proliferation and migration, since such features are required in tissue development and remodeling. Their biological characteristics are quite similar to those of cancer cells. Thus, cancer cells can be generated directly from fetal cells which already possess cancerous characteristics without undergoing further differentiation and de-differentiation processes. This shortcut is fetal cell carcinogenesis (Fig.1C) (24-26). A cancer cell is generated not by transformation but by proliferation without
differentiation and it shows cancerous characteristics simply because it reflects the biological characteristics of the originated fetal cell. In fetal cell carcinogenesis, a cancer cell does not look like Godzilla but looks like a Coelacanth, an ancient fish that has survived for 400 million years. The Coelacanth’s appearance differs greatly from that of other fishes, however it has not undergone transformation but has remained the same for many years, while other fishes have undergone gradual changes of their morphology.

**Fetal thyroid cell carcinogenesis**

Fig. 2 summarizes the fetal thyroid cell carcinogenesis model proposed recently (27). Thyroid tumor cells are generated directly from one of the three types of fetal thyroid cells by proliferation without differentiation. Tumor cells derived from TSCs and thyroblasts possess cancerous characteristics, since they reflect the biological characteristics of their origin. Anaplastic carcinomas are derived from TSCs. Papillary and follicular carcinomas are mainly derived from thyroblasts. Follicular adenomas are derived from prothyrocytes, which do not possess cancerous characteristics.

![Figure 2: Fetal thyroid cell carcinogenesis](image)

Any events that prevent fetal thyroid cells from differentiation can be a cause of cancer. It is suggested that RET/PTC or PAX8-PPARγ1 rearrangements and mutations in BRAF contribute to such events. In this model, these oncogenes act as an initiation but not promotion factor in thyroid carcinogenesis, which indicates that, unlike the classical multi-step carcinogenesis model in which activation of oncogenes is inevitable in tumor progression, this model does not assure the tumor suppression effect of the inhibitors against these oncogenes. At least in the thyroid, it is not likely that malignant transformation or dedifferentiation, which is described in multi-step carcinogenesis, takes part in the process of carcinogenesis, since if so, an increase in thyroid cancer risk should be observed in adults after radiation exposure, and an oncogene can induce thyroid cancer in adult mice. In fetal cell carcinogenesis, cancer is regarded as an abnormal development of fetal thyroid cells.

The very first event in thyroid carcinogenesis, prevention of fetal thyroid cells from differentiation, occurs before age 5. However, such an event does not become evident until middle age since the growth of fetal thyroid cells is very slow. Thyroid cancer becomes evident earlier when the environment is preferable for fetal thyroid cells. This explains why the prevalence of differentiated thyroid carcinoma is high in middle-aged women, since their origins are fetal thyroid cells existing mainly during the gestational period and fetal thyroid cells are likely to favor an environment exposed to estrogen (28).

Some recent reports support the above hypothesis. In an observational trial of papillary thyroid microcarcinoma, the proportion of patients whose carcinomas showed enlargement by 3 mm was only 15.9 %, even for a 10-year follow-up, which has confirmed their slow growth (29). Children under 18 years old in Fukushima, Japan, were subjected to the repeated mass screening of thyroid tumor with ultrasonography after the Fukushima nuclear plant accident in 2011. Although the scientific evidence denies the effect of radiation on their health status, the prevalence of abnormal findings in the thyroid was unexpectedly high and 90 of 295,511 children were suspected to have a malignant tumor in the thyroid by ultrasonography and aspiration biopsy cytology (the report from Fukushima Health Management Survey, May 19, 2014). These results have suggested that small thyroid carcinomas already exist in a considerable number of children and a part of them grow slowly to become clinical cancer in the middle age.

Differentiated thyroid carcinomas generally show a favorable prognosis, since they show slow growth, whereas local or distant metastases are observed at a high frequency (28). These features link directly to those of fetal thyroid cells, especially thyroblasts, which proliferate slowly but migrate rapidly. On the contrary, anaplastic carcinomas which are assumed to be derived from TSCs show poor prognosis due to rapid growth. This may reflect the biological characteristics of stem cells, which have an ability of unlimited proliferation.

Papillary thyroid microcarcinomas are observed in a high prevalence of up to 30 % of autopsied cases (30). At least some of these cells may not be carcinomas, but rather the remnants of thyroblasts.

**New concepts in fetal cell carcinogenesis**

**Reverse approach**

In fetal cell carcinogenesis, tumor cells arise directly from their corresponding fetal cell, consequently both resembling each other in biological characteristics. We can estimate the biological characteristics of fetal thyroid cells on which information is usually scarce, using the evidence obtained from thyroid tumors. This method is called the reverse approach.

For example, we can estimate the existing period of each predicted fetal thyroid cell. Poorly differentiated fetal thyroid cells, especially TSCs and thyroblasts, are not likely to exist abundantly in the thyroid in adults, since the adult thyroid usually does not regenerate after partial thyroidectomy (31). The fact that no induction of thyroid carcinomas by radiation in adults supports this assumption. After the Chernobyl accident, pediatric papillary carcinoma was most frequent at age 0 at the time of the accident, and the frequency rapidly decreased until age 5 (14). Follicle formation, which is a sign of the dominant existence of prothyrocytes, is first observed at approximately the 10th gestational week (32). These data suggest that thyroblasts are dominant up to the 10th gestational week, and then their number rapidly decreases, resulting in almost complete loss at age 5. The period of the existence of TSCs is not clear. However, when considering that no pediatric anaplastic carcinoma has been reported after the Chernobyl accident, it is likely that TSCs disappear or stop producing thyroblasts in the very early stage of pregnancy before the fetal thyroid starts iodine uptake. Remnants of TSCs might rarely exist in adults, explaining the rarity of anaplastic carcinoma.
Stem cell crisis

The reverse approach suggests TSCs and thyroblasts usually disappear before adolescence. This assumption leads to another question. Why do anaplastic carcinomas, which are considered to be derived from TSCs, occur only in the elderly? If anaplastic carcinomas are generated from the remnant of fetal thyroid cells after remaining silent for several decades, their origin must be TSCs, since stem cells are the only cells that are able to remain alive for many years without proliferation except for those with a special function such as germ cells, myocondrocytes and neurons (33). Furthermore, the co-existence of a differentiated component is not surprising, since TSCs have the ability to generate thyroblasts and prothyrocytes. When TSCs generate thyroblasts or prothyrocytes, the proliferating tumors act as differentiated tumors, whereas when TSCs themselves start to proliferate, the resulting tumor acts as an undifferentiated carcinoma and is recognized as an anaplastic transformation. One possible explanation for their sudden proliferation in the elderly is that TSC remnants cannot maintain the characteristics of a stem cell after many years of repeated proliferation, and begin uncontrolled proliferation as undifferentiated tumor cells, like a time-bomb (stem cell crisis) (26). This concept suggests an important assumption that simply aged but not transformed stem cells might be a source of poorly differentiated cancer. However, the fate of aged stem cells is not well known at present and is still controversial (34).

Mature cancer and immature cancer

One of the distinguishing features of thyroid cancer is its favorable prognosis in the young children (35). Thyroid carcinomas in the young children seem to be self-limiting, even though invasion or metastasis is observed frequently. Todaro et al. found that the tumorigenic capacity in differentiated thyroid cancer is confined to a small subpopulation of stem-like cells with high aldehyde dehydrogenase (ALDH) activity (36). These cells can be regarded as thyroid cancer-initiating cells (TCICs). On the contrary, ALDH-negative components in thyroid tumors have only limited proliferation ability, thereby tumors are mainly formed by the proliferation of TCICs.

These data have suggested that there are two types of differentiated thyroid carcinoma: one containing TCICs (immature cancer) and one that does not (mature cancer) (27). TCICs, which express stemness characteristics, are possibly derived from TSCs. Tumor cells derived from thyroblasts start proliferating immediately but slowly, whereas those derived from TSCs tend to stay quiescent for a while. Thus, tumors derived from thyroblasts (mature cancers) become clinically evident earlier than those from TSCs (immature cancers) resulting in their higher prevalence in the young children. Immature cancers show a high self-renewal activity resulting in the poor prognosis. This speculation is supported by the fact that anaplastic carcinomas, which are thought to be derived from TSCs in fetal cell carcinogenesis, are observed only in the elderly.

Cancer stem cells and fetal cell carcinogenesis

Cancer stem cells (CSCs) are defined as cancer cells persisting in tumors as a distinct population that possess characteristics associated with normal stem cells, and CSCs may generate tumors through the stem cell processes of unlimited proliferation, self-renewal and differentiation into multiple cell types. CSCs have been found in many types of cancer tissue and are believed to be related to poor prognosis (33).

Unfortunately, confusing descriptions about the relationship between the fetal cell carcinogenesis and CSC theory are often presented in some recent papers (37). The main part of the CSC theory is the existence of stem-like cells in a tumor tissue suggested to be derived from normal stem cells. A careful look at Fig. 2 makes us notice that the CSC theory describes the very beginning of the fetal thyroid cell carcinogenesis, in which the remnant of TSCs produces either anaplastic carcinoma cells or differentiated carcinoma cells. However, since thyroid tumors include various histological subtypes derived from many types of cells with different stages of differentiation, the entire mechanism of thyroid carcinogenesis cannot be explained only with TSCs or TCICs. Therefore, at least in the thyroid, the CSC theory alone is not sufficient to explain carcinogenesis.

It should be noted that CICs are also described in the multi-step carcinogenesis hypothesis (38, 39). However, there is a fundamental difference in their nature. In fetal cell carcinogenesis, CICs are generated from undifferentiated fetal cells, mainly stem cells, by preventing their differentiation, and the nature of CICs is a mirror image of their original fetal cells, thus, as described previously, aged “normal” stem cells can produce CICs. In contrast, in multi-step carcinogenesis, CICs are generated from stem cells by malignant transformation by accumulating damage in their genome, resulting in the acquisition of numerous malignant characteristics. Thus, CICs are transformed cells whose nature is quite different from stem cells. These considerations are important when we try to treat cancer, since therapeutic strategies targeting CSCs may or may not be harmful to normal stem cells in the fetal cell and multi-step carcinogenesis, respectively.

Diagnostic and therapeutic strategies based on fetal cell carcinogenesis

In most of the previous studies, thyrocytes or thyroid cell lines were used to clarify the mechanism of thyroid carcinogenesis, since these cells can be easily obtained. However, considering fetal cell carcinogenesis, the results in such studies might differ from what occurs in actual carcinogenesis, since events related to carcinogenesis occur only in fetal cells but not in differentiated cells. Even though it is quite difficult, we should not hesitate to obtain and use fetal thyroid cells in thyroid cancer research. Recent advance in the study of thyroid regeneration will support such a project.

In fetal cell carcinogenesis, a tumor is produced when undifferentiated fetal cells turn into multiple differentiated cells. Thus as described in the Section on mature cancer and immature cancer, the possibility remains that we sometimes misunderstand the exact biological characteristics of a tumor since we cannot determine whether a tumor contains a minor population of undifferentiated fetal cells or not. We should remember that mass analysis such as gene expression profiling, whole genomic scan, and proteomics analysis may have a definite limitation since they can only provide information based on a majority of cells. Intensive effort should be made in establishing a technology to detect the small number of fetal cells in the tissue in vitro or in vivo.

Acknowledgement
References


