Molecular Classification of Thyroid Tumor: A Proposal Based on the Fetal Cell Carcinogenesis Hypothesis

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Abstract

Recent experimental and clinical evidence in thyroid cancer has revealed its striking and puzzling features, attracting more attention to the novel concept of thyroid carcinogenesis, fetal cell carcinogenesis. In the fetal cell carcinogenesis hypothesis, thyroid tumors are derived from three types of thyroid fetal cells, and the characteristics of each tumor are totally dependent on its origin. Follicular tumors are classified into three groups based on gene expression profile. Follicular adenomas and carcinomas consist of one and two groups, respectively. Thus, benign tumors can be distinguished from malignant ones by measuring the expression of genes in follicular adenomas, such as TFF3. Malignant tumors are classified into mature and immature groups based on the existence of thyroid cancer stem cells. Immature cancer, suggested to be derived from thyroid stem cells, is associated with a poor prognosis due to its unlimited proliferation ability.

Keywords: Thyroid cancer stem cell, immature cancer, gene expression profile, papillary thyroid microcarcinoma

Recent evidence reveals striking features of thyroid cancer

Cancer has long been believed to be generated by multi-step carcinogenesis or an adenoma-carcinoma sequence. In multi-step carcinogenesis, a cancer cell is derived from a normal cell which does not possess cancerous characteristics. Such a cell acquires malignant characteristics such as invasion or unlimited proliferation ability via multiple sites of damage in its genome during proliferation. In thyroid, normal thyroid follicular cells are believed to transform into thyroid cancer cells in the middle age due to the altered activities of oncogenes and anti-oncogenes (1).

However, recent evidence on thyroid carcinoma has revealed a completely different feature of thyroid carcinogenesis. In 2011, Japan suffered from the East Japan earthquake followed by the Fukushima nuclear plant accident. Immediately after the accident, Fukushima health management survey was initiated with ultrasonographic screening performed in children under 18 years old. Surprisingly, 110 in the 297,046 children were diagnosed with papillary carcinoma (2). The prevalence was calculated to be approximately one out of 2,700, which was surprisingly high since juvenile thyroid carcinomas were believed to be rare before the data of this survey were published. It is clear that these carcinomas are not related to the radiation since the survey was started immediately after the accident. Thus, we have to draw the conclusion that the prevalence of small thyroid carcinoma is high, even in children.

Another surprising finding also came from Japan. In Japan, patients with small papillary carcinoma (papillary thyroid microcarcinoma, PTMC) that is less than 1 cm in diameter and without evident local or distant metastasis tend to be recommended to undergo follow-up involving repeated ultrasonography rather than immediate surgical resection. Ito et al. summarized the data from an observation trial (3). They followed 1,235 patients of up to 18 years. They observed the size of the PTMCs by ultrasonography and calculated the percentage of PTMCs with enlargement by 3 mm or more. To our surprise, only 8% of the PTMCs showed enlargement by 3 mm or more even after 10 years. These results have revealed that the growth rate of PTMCs is extremely low, probably much lower than we had expected before the observation trial. An important conclusion drawn from these facts is that the initiation of PTMCs occurs at least before the infantile period, since they appear as a tumor around 1 cm in diameter in middle age.

Several other sources of clinical and experimental evidence support the idea that thyroid carcinomas are derived from thyroid follicular cells but from something that exists only in infants. After the Chernobyl nuclear plant accident, the risk of thyroid cancer increased mainly in children under 5 years old (4), whereas 131I administration to adult patients with Graves’ disease does not result in an increased risk of thyroid cancer (5). Furthermore, the introduction of an oncogene to the thyroid gland of mice can produce thyroid cancer when it is introduced only during the fetal period, but not in adults (6-8).

One of our general recognitions about cancer cells is that they alter their biological characteristics according to their growth, and we refer to such a phenomenon as progression (9). It is believed that, after repeated proliferation, tumor cells acquire malignant characteristics such as abilities to invade and metastasize, and their growth rate increases gradually until they acquire an unlimited proliferation ability. In the observation trial by Ito et al., the patients were classified into three groups according to their ages: age equal to or over 60; age between 40-59; and age under 40. The growth rates of PTMCs were compared among these groups (3). In contrast to the general recognition, the growth rate of PTMCs was the lowest in the oldest patients and highest in the youngest patients, which suggested that these tumors showed rapid growth in the young but stopped growing in the old, and then showed no further progression.
The conclusion that PMTCs show no progression is supported by the following evidence. Firstly, in the above study by Ito et al., among the 1,235 patients, no patient died from thyroid cancer (3). Secondly, the rate of thyroid cancer in Korea has increased rapidly since 2000. This has been due to the introduction of ultrasonography and because the patients with even a small papillary carcinoma have undergone surgery. However, the increasing rates of thyroid cancer and related surgery did not result in a decrease in thyroid-cancer-related mortality (10). These results clearly indicated that the death due to thyroid cancer is not caused by the progression of small thyroid carcinomas. PMTCs are not likely to show progression and do not turn into lethal thyroid cancer. In other words, these results suggest that the first clinical appearance of a lethal thyroid cancer is not a PMTC but a large tumor or a tumor with local or distant metastasis, reflecting its rapid growth and malignant characteristics even in the initial stage.

There are two types of differentiated thyroid carcinoma. One type is mature cancer (Figure 1A). The initiation of mature cancer occurs at least at age 5. The tumor cells start proliferation immediately after tumorigenesis. Tumor cells proliferate rapidly in the young, and the tumor becomes clinically evident in middle age, mostly as PMTC. However, the tumor subsequently stops growing and shows no further progression, resulting in a favorable prognosis. Another is immature cancer (Figure 1B). As in mature cancer, the initiation of immature cancer occurs at least at age 5. However, for some unknown reason, the tumor cells remain silent until middle age. Then, after the middle age, they start proliferating suddenly and rapidly, leading to detection as a clinical tumor, mostly as a large tumor. The tumor cells show unlimited proliferation, resulting in a poor prognosis. The prognosis associated with thyroid carcinoma is much more favorable than those related to most cancers from other organs. This is simply because majority of the thyroid carcinomas are mature cancers, which do not cause cancer death. In other words, the prognosis associated with immature thyroid cancer alone is no better than those related to cancers from other organs.

The above consideration reveals some striking features of thyroid carcinomas, completely contradicting long held beliefs in multi-step carcinogenesis (11). Firstly, their origin is not from “normal” cells, thyroid follicular cells, as observed in adults. Secondly, tumor cells do not show progression; in other words, the origins of malignant and benign cells are different. Recent evidence from molecular-based analysis has revealed that several genetic abnormalities, such as RET/PTC and PAX8-PPARγ1, are observed at a high prevalence in differentiated carcinomas but not in poorly differentiated or anaplastic carcinomas, and the prevalence of BRAF gene mutation is much lower in anaplastic than in papillary carcinomas (12-14). These results clearly contradict the classical concept of multi-step carcinogenesis in which benign tumor cells become malignant due to accumulating genetic abnormalities in their genome.

Since recent data on thyroid carcinoma have been quite puzzling from the viewpoint of multi-step carcinogenesis, the emerging concept of thyroid carcinogenesis, fetal cell carcinogenesis, is now supported by many researchers (15-19). Figure 2 summarizes the basic concept of fetal cell carcinogenesis. In fetal cell carcinogenesis, thyroid cancer cells are derived not from thyroid follicular cells but from fetal thyroid cells which only exist until the infantile period. In the human thyroid, existence of three types of thyroid fetal cells has been suggested (20). Thyroid stem cells, which first appear in the pharynx during thyroid development, comprise a small number of undifferentiated cells that express thyroid transcription factor-1 (TTF-1, NKX-2.1) but not thyroglobulin (TG). They start moving to the front neck without evident proliferation as they change into thyroblasts. Thyroblasts express TTF-1 and TG and they continue moving to the front neck as they proliferate slowly. Histologically, they do not form follicles. As they settle at the front neck, the fetal thyroid starts forming a follicle. The fetal thyroid cells at this stage are called prothyrocytes. Prothyrocytes look similar to thyroid follicular cells but they lack the ability to produce thyroid hormone.

Anaplastic carcinomas are derived from thyroid stem cells, differentiated carcinomas (papillary and follicular carcinomas) are derived mainly from thyroblasts, and benign tumors such as follicular adenomas are derived from prothyrocytes. Any events that prevent these fetal cells from undergoing further differentiation can be a cause of carcinogenesis. For example, the mutated BRAF gene and RET/PTC produce papillary carcinomas by preventing thyroblasts from differentiating into prothyrocytes, and PAX8-PPARγ1 produces follicular tumors by preventing prothyrocytes from differentiating into thyrocytes.

There is a report supporting this hypothesis (21). Introduction of the mutated BRAF gene into the fetal thyroid of a mouse produced papillary carcinoma, and the mouse showed severe hypothyroidism due to a lack of thyroid follicular cells. Interestingly, the administration of a BRAF inhibitor restored thyroid follicles, which suggested that what looked like papillary carcinoma cells were actually thyroblasts, and they differentiated into thyroid follicular cells.

The most important feature of the fetal cell carcinogenesis hypothesis is that the characteristics of each tumor are totally dependent on its corresponding origin, i.e., the malignant features
of cancer cells are not acquired after repeated proliferation, but such characteristics are the reflection of the nature of their corresponding fetal cells. Thus, in fetal cell carcinogenesis, the most important information in order to identify the biological feature of a tumor is the type of the fetal cells it is derived from.

Considering the above, oncogenes take part mainly in tumor initiation but not in progression. Several studies reported that papillary carcinomas with the BRAF mutation were associated with a poor prognosis, whereas some other studies found no difference in the prognosis between papillary carcinomas with or without the BRAF mutation (22, 23). These controversial results can be partly explained by the possible existence of some pathological subtypes with a high or low prevalence of the BRAF mutation and with a different prognosis; thus, the use of different pathological criteria resulted in a different prognosis. In fact, the prevalence of the BRAF mutation is high in tall-cell and columnar variants of papillary carcinoma, which are associated with a poor prognosis, whereas the BRAF mutation is low in follicular variants of papillary carcinoma, which are sometimes difficult to distinguish from atypical follicular adenomas (24).

**Difference between mature and immature cancer**

In the fetal cell carcinogenesis hypothesis, cancer cells express malignant characteristics because their origins, immature fetal cells, possess such abilities. In the thyroid, tumors derived from two types of immature fetal cells, thyroid stem cells and thyroblasts, are considered to be malignant. A tumor arises when undifferentiated cells proliferate to produce many differentiated cells. Thus, differentiated carcinomas can be raised not only from thyroblasts, but also from thyroid stem cells. Tumors derived from thyroblasts and thyroid stem cells correspond to mature and immature cancers, respectively. Thus, immature cancer includes not only anaplastic carcinomas but also a part of differentiated carcinomas.

It should be noted that such a difference in origin will lead to a difference in the prognosis. Mature cancers derived from thyroblasts start proliferating immediately after initiation. However, their growth rate is not high, because their progenitors, thyroblasts, grow slowly during thyroid development. They become detectable in middle age but stop proliferating afterward due to their limited ability for proliferation. Thus, they tend to degenerate after surgery or radioisotope therapy, resulting in a favorable prognosis (Figure 3).

Immature cancers derived from thyroid stem cells stay dormant for decades after initiation. Stem cells after initiation may be regarded as thyroid cancer stem cells (TCSCs). They suddenly start proliferating after middle age, producing differentiated tumor cells which are similar to thyroblasts. Since tumor cells that are derived from stem cells possess an ability to undergo rapid and unlimited proliferation, the tumors grow back after surgery, and radioisotope therapy is not effective since undifferentiated cells do not take up radioactive iodine. Furthermore, when TCSCs themselves start to proliferate, the tumor looks like a cluster of undifferentiated tumor cells. This phenomenon is regarded as anaplastic transformation in multi-step carcinogenesis and called stem cell crisis in fetal cell carcinogenesis. Thus, the prognosis of patients with immature cancer is very poor.

Probably, the clinical course of patients with immature cancer is closely related to the nature of stem cells. Stem cells can remain dormant for a long period without proliferating unless necessary, but once they start to proliferate, they can produce unlimited numbers of differentiated cells (25). Thyroid carcinomas induced by radioactive iodine are regarded as mature cancers originating from thyroblasts, since thyroid stem cells are not likely to take up iodine.

**Molecular-based classification of thyroid carcinoma**

There are many molecular markers that are useful in identifying anaplastic and papillary carcinomas. For example, anaplastic carcinomas are those that express oncofetal fibronectin (onfFN, FN1) but not TG, whereas papillary carcinomas express both onfFN and TG (26, 27). However, the situation is different in follicular carcinoma. Some previous studies presented data showing that diagnoses of thyroid follicular tumors using molecular-markers almost perfectly matched those on pathological examination (28-30). However, to date, no follow-up studies confirming such data have been published.

Taniguchi et al. analyzed the expression levels of more than 2,500 genes expressed in follicular tumors to examine any genes or sets of genes that can differentiate follicular carcinomas from...
adenoma (31). However, the diagnostic accuracy was never over 90% even when they used the combination of up to 60 genes, and the average accuracy remained at around 85%. These data suggest that the pathological diagnosis according to the present WHO criteria does not reflect the biological characteristics of follicular tumors in about 15% of the cases.

We reported this phenomenon as the “15% issue” in follicular thyroid carcinoma (32). There is some evidence supporting the existence of the 15% issue. Some studies reported that marked observer variation existed in the pathological diagnosis of thyroid follicular carcinoma (33, 34). Ito et al. reported that a distant metastasis was observed in 0.2% of the patients diagnosed with a benign tumor at surgery (35). Considering these data, it should be noted that regarding follicular carcinoma, we cannot expect a perfect match between molecular analysis and the pathology. Probably, it is time to revise the present pathological diagnostic criteria by introducing some information from molecular aspects.

Table 1: Genes that classify thyroid follicular tumors

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<tr>
<th>Gene Group A</th>
<th>Gene Group B</th>
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<tr>
<td>ANXA1, C13 or f33, CYP1B1, FAP, FN1, IL17RD, PDLM14, RUNX2, TIMP1</td>
<td>AIF1L, CDH16, FAM162B, FGFR2, GJB6, KCNJ13, KIAA1467, SLC25A15, TFCP2L1, TF3, TMEM171</td>
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Recently, Shimizu et al. analyzed gene expression profiles of follicular tumors using DNA microarrays, and found that follicular tumors could be classified into three groups by two sets of genes (36). A Polish group reported similar results (37). One group consisted of mainly follicular adenomas and the other two groups consisted of mainly follicular carcinomas. The follicular adenoma group expressed gene group B. One follicular carcinoma group expressed gene group A, but not gene group B (Table 1). The other follicular carcinoma group did not express either gene group. These studies raise a question about the reliability of previous reports in which follicular tumors were diagnosed with a high accuracy using a gene overexpressed in follicular carcinomas (28, 38, 39).

Interestingly, gene group A contained several genes known to be overexpressed in papillary carcinomas, such as FN1, RUNX2, and TIMP1, which may be marker genes of thyroblasts (26, 40, 41). These results support the hypothesis that not only papillary but also follicular carcinomas are derived from thyroblasts, and thus follicular tumors expressing these genes can be regarded as papillary-type follicular carcinoma. In gene group B, there are some genes overexpressed in the normal thyroid, such as CDH16, FGFR2, and TF3 (42-44). These genes might be differentiation markers expressed in prothyrocytes (Figure 2). Follicular carcinomas that express none of these genes are regarded as non-papillary-type follicular carcinomas, and they might be differentiated from papillary carcinomas or papillary-type follicular carcinomas.

The simplest diagnosis of thyroid tumors to identify malignant tumors is to measure the expression levels of the genes in gene group B, because tumors expressing these genes are definitely benign since they are derived from prothyrocytes that do not possess malignant characteristics. Among the genes in gene group B, TF3 is the most promising in the preoperative diagnosis using thyroid aspirates, since its expression level in follicular tumors is high enough for accurate quantitative measurement of its copy number in a small number of cells (44). In fact, such a TF3-based diagnosis is now undergoing trials in many laboratories (45-47).

**Challenge in diagnosing lethal thyroid carcinoma**

In this way, a molecular-based diagnosis to differentiate types of malignant thyroid tumors will be established in the near future. However, when we take fetal cell carcinogenesis into account, the differentiation of lethal carcinomas seems to be quite difficult at present. As described above, immature but not mature cancer is lethal. However, the differentiation between the two cancers is not always easy in pathology. In differentiated carcinomas, the difference between the two lies in whether a specimen contains a small number of undifferentiated tumor cells, i.e., TCSCs or not. However, the number of TCSCs is usually very small, making them hard to detect with pathological techniques.

Some recent studies have proved the existence of TCSCs in differentiated carcinomas, and some methods to detect TCSCs were introduced (48, 49). Flow cytometry is a method widely used for the detection of stem cells or cancer stem cells. However, some difficulties must be overcome before applying flow cytometry clinically. Firstly, no definite marker to identify TCSCs have been established. There have been some studies referring to possible markers for TSCSs, but both the target molecules and percentages of TSCSs in tumor tissues differed greatly among studies. Secondly, sample preparation is quite difficult, since flow cytometry requires a large number of freshly digested single cells from tumor tissue. Another method, use of nude mouse implantation to estimate tumorigenicity, is even more difficult. It requires dispersed living cells under sterile conditions and takes at least several weeks to obtain results.

**Classification of thyroid tumors based on the fetal cell carcinogenesis hypothesis**

Figure 4 shows the proposed classification of thyroid tumors based on fetal cell carcinogenesis. Thyroid tumors are derived from three types of fetal thyroid cells. Tumors from prothyrocytes are benign and they express genes in gene group B such as TF3. Malignant tumors from thyroid stem cells or thyroblasts do not express these genes, but most of them express thyroblast- or thyroid stem cell-specific genes represented by genes in gene group A such as FN1. Malignant tumors are further classified into mature and immature cancers based on the existence of TSCSs. The occurrence of cancer death is limited in immature cancer which shows the ability of unlimited proliferation.
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Conflicts of Interest: None

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