Original Article

Immunohistochemical expressions of fatty acid synthase and phosphorylated c-Met in thyroid carcinomas of follicular origin

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Abstract: Thyroid carcinoma is the most common endocrine malignancy and the first cause of death among endocrine cancers. Fatty acid synthase (FASN) and c-Met are overexpressed in many types of human cancers. Recent studies have suggested a functional interaction between FASN and c-Met. However, their roles in thyroid carcinomas have not been fully investigated. In this study, we evaluated the expressions of FASN and phosphorylated (p)-c-Met by using immunohistochemistry in thyroid carcinomas of follicular origin, from 32 patients. The adjacent non-neoplastic thyroid tissue was also evaluated for comparison. Immunoreactive intensity and extensiveness were semi-quantified. The overexpression of FASN was observed in a subset of papillary thyroid carcinomas (PTC) including the classical type and tall cell, follicular, trabecular/insular and diffuse sclerosing variants, a subset of follicular thyroid carcinomas (FTC), and the PTC and FTC components in anaplastic thyroid carcinomas (ATC). No overexpression was observed in the ATCs per se and the columnar cell, solid, and cribriform variants of PTCs. All Hürthle cell variant FTCs and non-neoplastic Hürthle cells demonstrated positive staining for FASN while the non-neoplastic follicular cells without Hürthle cell change were negative. An association in overexpression between FASN and p-c-Met was observed in the majority of carcinomas as well as in the non-neoplastic Hürthle cells. In conclusion, overexpressions of FASN and p-c-Met were observed in a subset of thyroid carcinomas of follicular origin, which may be of values for targeted therapy and predicting prognosis while the positive immunostaining for these immunomarkers may be non-specific for Hürthle cell thyroid carcinomas.

Keywords: C-Met, fatty acid synthase, immunohistochemistry, thyroid carcinoma

Introduction

Fatty acid synthase (FASN) and c-Met have been increasingly studied as potential therapeutic targets in human cancers; however, their roles in thyroid carcinomas have not been fully elucidated.

Human FASN is an enzyme catalyzing syntheses of fatty acids. Recently it has been further divided into two types: type I and type II. The type I FASN is a 270-kDa cytosolic enzyme, a key enzyme catalyzing the synthesis of long-chain saturated fatty acids [1-3]. The type II FASN produces the fatty acids that play important roles in the mitochondrial function [4].

Normally, free fatty acids come from diet and de novo synthesis catalyzed by Type I FASN in lipogenic tissues. The level of FASN expression in the non-neoplastic cells is low because the cells preferentially use circulating free fatty acids from diet. Recently, FASN has been rediscovered as a marker for cancers because the type I FASN has been shown to have oncogenic activity [5]. The expression of FASN in neoplastic cells is up-regulated by de novo synthesis via multiple steps including gene amplification, transcription, translation and post-translational modifications and confers growth and survival advantages in many types of cancers [6]. It has been demonstrated that the overexpression of FASN causes resistance to both chemotherapy and radiation therapy in human cancers [7, 8]. While the FASN overexpression has been studied in various human cancers, the role of FASN
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in thyroid cancers has not been fully investigated [9]. There are only two previous studies published in the literature including one study of papillary thyroid carcinoma and the other one using cultured anaplastic thyroid carcinoma cell lines [10, 11]. Recent studies have suggested a functional interaction between FASN and some receptor tyrosine kinases for the promotion of tumorigenesis in tumors [12, 13]. c-Met is one of the receptor tyrosine kinases that has been implicated in playing a major role in tumorigenesis. c-Met is a proto-oncogene that encodes a protein known as hepatocyte growth factor receptor (HGFR) [14, 15]. The c-Met protein (HGFR) possesses tyrosine-kinase activity and its activation triggers tumor growth, angiogenesis and tumor metastasis [16]. c-Met expression has been demonstrated in thyroid cancers [17-21, 22, 23]. Some of the studies also indicate that the c-Met activation implicates aggressive behaviors including tumor invasiveness, metastasis and chemoradioresistance in cultured human thyroid cancer stem cells and PTCs [19-21, 22]. However, to our knowledge, the association of c-Met with FASN in thyroid cancers has not been reported.

Several FASN inhibitors including some commonly used drugs for diabetics and weight loss have shown to induce significant anti-tumor activity in human breast, endometrial, prostate, ovary, colon and mesothelial malignant neoplasms cell lines and xenografts [24, 25]. The inhibition of FASN appears to selectively kill neoplastic cells with minimal side effects to non-neoplastic cells, which makes FASN a potential good therapeutic target [7, 26-31]. A variety of c-Met pathway antagonists with potential clinical applications have also been investigated, which include both monoclonal antibodies and small-molecule tyrosine kinase inhibitors [32]. Therefore, it becomes more desirable than ever to define the roles of FASN and c-Met in thyroid cancers.

In the present study, we used immunohistochemistry to investigate expressions of FASN and the activated c-Met in a spectrum of thyroid carcinomas of follicular origin.

Materials and methods

Archival thyroidectomy specimens were obtained from 32 patients with thyroid carcinomas of follicular origin including 22 papillary thyroid carcinomas (PTC: 6 classical type and 6 follicular, 4 tall cell, 2 columnar cell, 1 diffuse sclerosing, 1 trabecular/insular, 1 solid and 1 cribriform variants), 8 follicular thyroid carcinomas (FTC: 3 conventional type and 5 Hurthle cell variant) and 2 anaplastic thyroid carcinomas (ATC: 1 with contiguous PTC and 1 with FTC). The protocol of this study was approved by the Institutional Review Board of the University of Texas Health Science Center at Houston.

Immunohistochemical staining

Immunohistochemical stains were performed on formalin-fixed and paraffin-embedded unstained sections of 4 µm thickness. Fatty acid synthase rabbit monoclonal antibodies (dilution 1:50, Cell Signaling Technology, Beverly, MA) and phospho-Met (Tyr1234/1235) (p-c-Met) rabbit monoclonal antibodies (dilution 1:100, Cell Signaling Technology) were utilized for immunohistochemical staining. The unstained sections were deparaffinized in xylene and rehydrated in a graded series of ethanol. Heat-induced epitope retrieval was performed. Endogenous pigments were quenched with 3% H₂O₂ in methanol for 10 minutes and rinsed with Tris Buffered Saline with Tween 20 (TBST). The remaining procedure took place on a DAKO Autostainer programmed to treat each slide with primary antibodies and with incubation at room temperature for one hour. VECTASTAIN Elite ABC Kit (Rabbit IgG) PK-6101 (Vector Laboratories, Inc. Burlingame, CA) was used. The slides were rinsed and incubated with DAB (3,3’-diaminobenzidine chromogen solution) for 10 minutes. The slides were rinsed again and counterstained with Mayer’s hematoxylin, treated with xylene, and cover-slipped. Appropriate positive and negative controls for each case were obtained.

Assessment of immunohistochemical staining

Chromogenic signal was assessed by bright-field microscopy. Both staining intensity and extensiveness were evaluated. Staining intensity was graded as negative (0), weak (1+), moderate (2+), and strong (3+). Staining extensiveness was the percentage of tumor cells positively stained with a range from 0% to 100%. The overexpression was defined as a tumor with positive staining (1+ to 3+) in 10%
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Results

The expression of FASN was present in the cytoplasm. The overexpression was identified in 17 out of 32 cases including 9 of 22 PTCs (3/6 classical type and 1/6 follicular, 1/1 trabecular/insular, 1/1 diffuse sclerosing and 3/4 tall cell variants), 6 out of 8 FTCs (1/3 conventional type and 5/5 Hürthle cell variant) and 2/2 contiguous well differentiated thyroid carcinoma components of the ATCs (PTC component in one case and FTC component in the other case). There was no overexpression of FASN in the anaplastic component in both ATCs and 2/2 columnar cell, 1/1 cribriform and 1/1 solid variants of PTCs. The non-neoplastic thyroid follicular cells were negative for FASN except the follicular cells with Hürthle cell change. The expression of p-c-Met was cytoplasmic and focally plasmalemmal. The overexpression was associated with that of FASN in both carcinoma tissue and the non-neoplastic Hürthle cells except one FASN negative columnar cell variant PTC that was weakly positive for p-c-Met. The expressions of FASN and p-c-Met are summarized in Table 1. Examples of overexpressions of FASN and p-c-Met in the thyroid carcinomas are demonstrated in Figures 1, 2 and 3.

Discussion

Our study demonstrates: (1) overexpressions of fatty acid synthase (FASN) and phosphorylated (p)-c-Met in a subset of PTCs and FTCs and all well differentiated thyroid carcinoma (PTC and FTC) components in ATCs, (2) no overexpressions of both FASN and p-c-Met in ATCs per se, (3) overexpressions of FASN and p-c-Met in all Hürthle cell variant FTCs as well as in non-neoplastic Hürthle cells, and (4) an association in overexpression between FASN and p-c-Met. The oncogenic mechanisms of FASN have been under investigation since it was found that fatty acids synthesized by FASN in cancer cells were not only used for cellular membrane construction but also involved in the activation of oncogenic signaling pathways. The most attention has been drawn to the PI3K/Akt signaling pathway, one of the oncogenic signaling pathways, known to play an important role in cancer cell survival and resistance to chemoradiation therapy. Studies have demonstrated that the expression of FASN is linked with the PI3K/Akt signaling pathway in cancers of ovary, liver, prostate, colorectum and breast [33-37]. Recently, Uddin et al demonstrated that FASN was overexpressed in a subset of papillary thyroid carcinomas including classical type, follicular variant and tall cell variant and that the FASN

Table 1. Immunohistochemical overexpressions of FASN and p-c-Met in thyroid carcinomas of follicular origin

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>subclassification</th>
<th>FASN (positive/total cases)</th>
<th>p-c-Met (positive/total cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTC</td>
<td>Classical type</td>
<td>3/6</td>
<td>3/6</td>
</tr>
<tr>
<td></td>
<td>Follicular variant</td>
<td>1/6</td>
<td>1/6</td>
</tr>
<tr>
<td></td>
<td>Cribriform variant</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>Trabecular/insular variant</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>Solid variant</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>Diffuse sclerosing variant</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>Columnar cell variant</td>
<td>0/2</td>
<td>1/2</td>
</tr>
<tr>
<td></td>
<td>Tall cell variant</td>
<td>3/4</td>
<td>3/4</td>
</tr>
<tr>
<td>FTC</td>
<td>Conventional type</td>
<td>1/3</td>
<td>1/3</td>
</tr>
<tr>
<td></td>
<td>Hürthle cell type</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>ATC</td>
<td>Anaplastic component:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PTC component</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td>FTC component</td>
<td>1/1</td>
<td>1/1</td>
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<tr>
<td></td>
<td>Anaplastic component:</td>
<td></td>
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</tbody>
</table>
|                    | ATC: anaplastic thyroid carcinoma; FTC: follicular thyroid carcinoma; PTC: papillary thyroid carcinoma; p-c-Met: phosphorylated-c-Met
expression was not associated with patients’ age, gender, histology type, extrathyroidal extension, and cancer stage, but correlated with overexpression of p-Akt by immunostaining patients’ tissue on tissue microarray [11]. They also observed that the inhibition of FASN caused not only down-regulation of FASN but also inactivation of Akt activity. In accordance, one of our previous studies showed Akt activation in PTCs as well [38]. Our current study concurs with Uddin et al of the overexpression of FASN in a subset of PTCs. Collectively, these findings support the hypothesis of the link between FASN and Akt signaling pathways.

Moreover, emerging evidence has suggested that FASN is upstream of c-Met and that the inhibition of FASN resulting downregulation of c-Met expression has been observed, which suggests that the FASN inhibitors play an important role in the cancers with the activation of FASN – c-Met pathway [39, 40]. Furthermore, several studies suggested a strong pathogenic role of c-Met via the Akt signaling pathway in a variety of tumors [40-43]. Therefore, the oncogenic signaling pathway of FASN-c-Met-PI3K/Akt has been postulated [39].

Phosphorylated status of c-Met protein represents the activated state because the interaction of the c-Met protein (HGFR) with hepatocyte growth factor (HGF) results in autophosphorylation at multiple tyrosines, which recruit several downstream signaling components including

Figure 1. Immunohistochemical staining of fatty acid synthase (FASN) and phosphorylated (p)-c-Met showing cytoplasmic positivity in papillary thyroid carcinomas of classical type (A: H&E; B: FASN; C: p-c-Met) and tall cell variant (D: H&E; E: FASN; F: p-c-Met) and no overexpressions in columnar cell variant (G: H&E; H: FASN; I: p-c-Met) (x 400).
PI3K phosphorylation that subsequently activates Akt [44]. Phosphorylation of Tyr1234/1235 in the c-Met kinase domain is critical to its kinase activation [45]. The antibody for c-Met that we used in the current study is against phosphorylated c-Met protein and, thus, the immunoreactivity indicates the activated state of c-Met.

Our present study shows that the well differentiated thyroid carcinoma (PTC and FTC) components contiguous to ATCs overexpress both Fatty acid synthase, phosphorylated c-Met and thyroid carcinomas.
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The finding suggests that the well differentiated thyroid carcinomas with overexpressions of FASN and p-c-Met may be of aggressive nature. This postulation is supported by the observations reported in the literature that the high level of FASN expression is associated with poor prognosis, higher risk of recurrence, and shorter survival of human cancers of breast, prostate, lung, colon, ovary, endometrium, kidney, head and neck, sarcoma, melanoma and nephroblastoma [8, 46]. In addition to the interaction with c-Met, FASN has

**Figure 3.** Overexpressions of FASN and p-c-Met in Hürthle cell variant of follicular thyroid carcinoma (A: H&E; B: FASN; C: p-c-Met; x 400) and non-neoplastic Hürthle cells in lymphocytic thyroiditis (D: H&E, x 400; E: FASN, x 100; F: p-c-Met, x 100); no overexpressions of FASN and p-c-Met in non-neoplastic follicular cells without Hürthle cell change (left sides in E and F).
been found to be associated HER2 expression in breast carcinoma and higher Gleason grade in prostate cancer, respectively [35, 47]. The findings suggest that the activation of FASN may reflect a higher level of intrinsic aggressiveness in the cancers. The c-Met activation has also reported to implicate aggressive behaviors in many types of tumors including PTC [19, 21, 22]. Therefore, FASN and c-Met positive well differentiated thyroid carcinomas may have a more aggressive clinical behavior than FASN and c-Met negative well differentiated thyroid carcinomas.

In the present study, we also discover that ATC component per se does not overexpress FASN and p-c-Met. The finding coincides with the observation of Ruco LP et al [22]. They found that the immunoreactivity for Met protein was absent in two out of two ATCs. The consistent results of the absence of expressions of FASN and p-c-Met suggest that the tumorigenesis of this highly aggressive thyroid carcinoma is not due to the activation of FASN-c-Met pathway and may be signaled through other pathways, e.g. mTOR, especially mTORC2 pathway, for which the upstream regulators have not been defined, as demonstrated in our previous studies [38, 48].

In the present study, overexpressions of FASN and p-c-Met are identified in all the follicular carcinomas of Hürthle cell variant as well as non-neoplastic Hürthle cells while the non-neoplastic follicular cells without Hürthle cell change do not show overexpressions of both markers. These findings challenge the notation of FASN and p-c-Met overexpressions associating with an aggressive behavior in thyroid Hürthle cell carcinomas. To date, the clinical significance of the Hürthle cell carcinomas is still controversial. Some observations suggest that the Hürthle cell carcinomas have a more aggressive behavior and a poorer outcome compared with the conventional type follicular carcinoma [49-51] while growing evidence demonstrates that Hürthle cell carcinomas are not more aggressive than their conventional counterparts [52]. In the literature, there are only few studies that demonstrate the expression of c-Met protein in thyroid Hürthle cell carcinomas [22, 23] and no studies of FASN in thyroid Hürthle cell carcinomas found. Whether expressions of FASN and c-Met in Hürthle cell carcinomas are associated with an aggressive clinical course has not been reported. Our present study demonstrates the first observation of FASN overexpression associated with Hürthle cell morphology regardless of neoplastic or non-neoplastic process and the activated c-Met expression in neoplastic and non-neoplastic Hürthle cells that is consistent with the recent report of c-Met expression in Hashimoto’s thyroiditis by Ruggeri RM et al [32]. Taken together, the findings suggest that overexpressions of FASN and c-Met in Hürthle cell lesions are not always associated with an aggressive behavior. The mechanisms of expressions of these markers in Hürthle cells are not clear. In 2003, Zhang et al first demonstrated that human mitochondria contained type II FASN distinct from the type I FASN [4]. The type II FASN may play an important role in mitochondrial function. According to these discovery and as we know that both neoplastic and non-neoplastic Hürthle cells have cytoplasm packed with mitochondria [53], the overexpression of FASN in the neoplastic and non-neoplastic Hürthle cells demonstrated in our current study may be due to the activation of type II FASN in mitochondria or even other unknown mechanisms causing the non-specific staining as well. Therefore, the overexpression of FASN in the Hürthle cells may not be specific for the cytosolic type I FASN. Because c-Met is downstream of FASN, it may show associated expression. Thus, the interpretation of overexpressions of these markers in Hürthle cell lesions should be with caution. In addition, our results suggest that a precaution should be taken to prevent potential adverse effect on the mitochondrial type II FASN while targeting type I FASN for cancer therapy.

In conclusion, this study demonstrates overexpressions of FASN and p-c-Met in a subset of papillary and follicular thyroid carcinomas but not in anaplastic thyroid carcinoma. The positive immunostaining for FASN or p-c-Met may or may not represent specific oncogenic markers and therapeutic targets in thyroid Hürthle cell carcinomas. The overexpressions of FASN and p-c-Met in the precursor neoplasms (PTC and FTC) concomitant to ATCs may imply the aggressive nature of differentiated thyroid carcinomas with overexpressions of these markers. These findings may be of values for targeted therapy and predicting prognosis for thyroid carcinomas.

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