Abstract. In normal tissues, energy-providing lipids come principally from circulating lipids. However, in growing tumors, energy supply is mainly provided by lipids coming from de novo synthesis. It is not surprising to see elevated expression of several lipogenic genes in tumors from different origins. The role of lipogenic genes in the establishment of the primary tumor has been clearly established. A large number of studies demonstrate a role of fatty acid synthase in the activation of cell cycle and inhibition of apoptosis in tumor cells. Other lipogenic genes such as the acetyl CoA carboxylase (ACC) and the stearoyl CoA desaturase 1 (SCD1) are highly expressed in primary tumors and also appear to play a role in their development. However, the role of lipogenesis in the metastatic process is less clear. In the present review, we aim to present the most recent evidences for the key role of lipogenic enzymes in the metastatic process and in epithelial to mesenchymal transition.

Contents

1. Introduction
2. Lipogenesis, tumor growth and apoptosis
3. The metastatic process
4. Implication of fatty acid synthase
5. Implication of stearoyl CoA desaturase 1
6. Implication of other lipogenic genes
7. Conclusions

1. Introduction

Solid tumors require high levels of energy for growth and membrane synthesis. Lipids provide this energy. In normal tissues lipids come from circulating lipids, while cancer cells mainly use de novo synthesized lipids (1). As a result, the rate of lipogenesis is highly induced (2).

Lipogenesis occurs both in liver and adipose tissues resulting in the synthesis of de novo fatty acids from acetyl CoA synthesized by glycolysis (Fig. 1). Acetyl CoA is then carboxylated by ACC forming malonyl CoA. Malonyl CoA and acetyl CoA are further processed by fatty acid synthase (FAS) in palmitic acid, which is then transformed by Elvol6 into stearic acid (3). SCD1 catalyzes the formation of palmitoleoyl-CoA and oleoyl-CoA from palmitoyl-CoA and stearoyl-CoA, respectively (4), which are preferentially transformed in triglycerides for storage in adipose tissue or phospholipids for membrane formation (5).

Expressions of ACC, FAS and SCD1 are under the control of the transcription factors LXR and the sterol regulatory element-binding protein-1c (SREBP-1c) (3). The 5’ AMP-activated protein kinase (AMPK) has been implicated in the control of hepatic lipogenesis (6) through inactivation of ACC (7) and SREBP-1c (6). ACC and FAS are overexpressed in numerous types of cancers (2,8) while high levels of mono unsaturated fatty acids (MUFA) were found in tumors (9) as a result of increased SCD1 expression and activity. SREBP1 has also been implicated in tumor growth (10). Therefore, high rate of lipogenesis is probably associated with tumorogenesis. High lipogenic activity was also associated with cancer progression and metastasis (11). As suggested, high SREBP1 expression may explain at least in part the increased expression of lipogenic genes associated with stratification of the malignancy. However, independently of SREBP1, the lipogenic enzymes expression correlates with the state of malignancy. The most recent findings on the role of lipogenesis in cancer progression and metastatic process are presented.

2. Lipogenesis, tumor growth and apoptosis

An elevated FAS expression induces progression of cancer cells into S phase (12). In contrast, inhibition of FAS expression decreases tumor growth and it induces apoptosis of cancer cells (8,13). Elevated ACC expression is observed in the early stage of breast cancer (14) and silencing its expression results in growth inhibition and apoptosis of cancer cells (15-17). High SCD1 expression is associated with cancer cell proliferation (18) and with a decrease in cell death (18-21). SREBP1c also plays a role in the transformation of normal cells (22).

Lipogenesis is induced in cancer cells by EGF through activation of the HER2/neu receptor (23) and the PI3-kinase/Akt pathway (24-26) targeting SREBP-1 (24,27).
As AMPK inhibits ACC by phosphorylation, its inactivation diminishes lipid supply and blocks cell cycle decreasing cell division and tumor growth (28). Therefore, lipogenesis probably provides energy supply to cancer cells stimulating cell division and survival leading to tumor growth.

3. The metastatic process

Metastasis is a complex multi-step process. Tumor cells need to escape from the primary tumor and to enter in the blood or in the lymphatic system. Most of the circulating cells undergo apoptosis (29) but some of them survive and invade new tissues (30). Epithelial to mesenchymal transition (EMT) has been associated with tumor progression and metastasis (31,32). It dissolves tight junctions between epithelial cells, the extracellular matrix and the adherent basolateral junctions leading to a disorganized and mobile mesenchymal cell population (32).

One of the first events of EMT is the loss of E-cadherin, a transmembrane protein implicated in formation of the tight junctions (33). This is the result of increased expression of its transcriptional repressors (34,35). In normal conditions, GSK3β phosphorylates E-cadherin transcriptional repressors targeting them to proteasome degradation thus allowing transcription of E-cadherin (36) (Fig. 2). In cancers, activation of TGF-β, Wnt, RTK and integrin pathways (37,38) leads to inhibition of GSK3β (39). Consequently, E-cadherin transcriptional repressors are no longer phosphorylated nor degraded leading to inhibition of E-cadherin expression (40). Another consequence is the loss of β-catenin phosphorylation that cannot be targeted to the proteasome and accumulates in the cytosol. It further translocates to the nucleus where it activates the transcription of genes such as c-myc, an important cell cycle regulator (41,42).

E-cadherin is also implicated in the actin cytoskeleton organization. Its direct binding to actin filament or to β-catenin maintains cell polarity and tissue architecture (43). In cancer cells, once the E-cadherin/β-catenin complexes disappear, the actin network is disrupted modifying cell migration (44,45). Nuclear β-catenin will also increase expression of mesenchymal proteins (46,47). These molecular events will allow EMT inducing the migration and invasion of cancer cells and metastasis.

4. Implication of fatty acid synthase

Among the lipogenic enzymes implicated in the development of metastasis, FAS is certainly the most studied protein (Table I). Prognostic and survival of patients with cancer are mainly predicted by the presence of metastasis (48) and overexpression of these enzymes is a good marker of poor prognosis. However, the exact molecular role of FAS in EMT and cancer progression is less clear.
of FAS has been associated with poor prognostics in several hormone-dependent cancers (49-52). The direct association between FAS expression and metastasis has also been observed in prostate cancers (53) and breast carcinomas (49).

In the transgenic adenocarcinoma of the mouse prostate (TRAMP) model which closely mirrors the progression of prostate cancer observed in human, FAS expression and activity are high compared to control littermates (54). Injection of immunodeficient mice with human prostate cancer cells overexpressing FAS and the androgen receptor (AR) leads to invasive adenocarcinomas (55). Androgens stimulate FAS expression in prostate cancers (55) by increasing the nuclear levels of SREBP (57). This is probably the result of increased SCAP expression that exports SREBP from the endoplasmic reticulum to the Golgi where it is activated by cleavage. As a consequence of androgen action on SREBP, expression of lipogenic genes is increased (56,57). Downstream of the AR, the PI3K\Akt pathway has been implicated in FAS activation (58). In prostate cancer, the isopeptidase USP2a has been also implicated in the activation of FAS expression by inhibiting its proteosomal degradation (59).

In ovarian cancer cells, proteolysis degradation of FAS and focal adhesion kinase (FAK) cause a strong reduction of the vascular endothelial growth factor (VEGF)-mediated cell migration and invasion (60), in the same study, the isopeptidase USP2a was also shown to stabilize FAS. In breast cancer cells, the green tea extract EGCg causes accumulation of β-catenin in the cytosol and decreased expression of E-cadherin (61). EGCg appears to disturb cell adhesion by modifying FAS and the EGF receptor (EGFR) signaling pathway.

FAS has also been implicated in the transformation of breast cancer cells through an effect on the EGFR (HER2/neu isoform) expression (62). HER2/neu is a proto-oncogene associated with the development of metastasis in breast cancer (63). In HER2-positive cells, elevated FAS expression stabilizes the lipid rafts and consequently, HER2/neu expression is increased activating downstream signaling pathways (64). EGF also increases FAS transcription establishing a positive feedback loop between FAS and EGF (65-67).

FAS expression is stimulated by estrogen in both endometrial and breast cancer cells (51). However, this increase is probably associated with the establishment of the primary tumor as the presence of estrogen and progesterone receptors in tumors provide better prognosis for the patients than those expressing HER2/neu (63,68).

Correlative associations between FAS expression and poor prognosis for patients were also observed in non-hormone-dependent cancers (69-71) as well as with metastasis (72).

In metastatic renal cancer, FAS expression is strongly induced compared to non-transformed tumors (73). In human pancreatic cells, invasiveness was abolished by C75, a synthetic FAS inhibitor possibly through downregulation of

Figure 2. The epithelial to mesenchymal transition. In normal state, cells are associated together by a complex formed by E-cadherin, β-catenin and α-catenin. This complex interacts with actin filament stabilizing cell interaction. In these conditions, GSK3β is not phosphorylated and can phosphorylate β-catenin and E-cadherin transcriptional repressors. Consequently, the phosphorylated proteins are targeted for proteosomal degradation. In a transformed state, upon activation of TGF-β, Wnt, integrin and RTK signaling pathways, GSK3 is phosphorylated and inactivated. Consequently, β-catenin and E-cadherin transcriptional repressors are not degraded. Therefore, β-catenin translocates to the nucleus where it activates c-Myc transcription while E-cadherin expression is inhibited. This leads to destabilisation of the cell-cell adhesion complex and disruption of the actin filament network.
HER2/neu and/or STAT3 phosphorylation (72). In a mouse model of spontaneous melanoma metastasis, direct IP injection of Orlistat, a natural FAS inhibitor, inhibits metastasis in lymph nodes by more than 50% (75). In xenograft models of advanced colon cancer, inhibition of FAS decreased hepatic metastasis (76,77) implicating AKT downstream of FAS. Inhibition of FAS also attenuates the activation of the MET receptor and FAK, two proteins implicated in adhesion, migration and invasion of cancer cells (60).

The above studies point to a key role of FAS in cancer progression, probably through modulation of lipid raft formation leading to activation of EGFR, HER2/neu and MET. Consequently, downstream signaling pathways are activated increasing nuclear localization of SREBP1c activating FAS and other lipogenic genes describing a positive feedback loop.

5. Implication of stearoyl CoA desaturase 1

An increased content of MUFAs has been observed in transformed cells suggesting a role for SCD1 in tumorigenesis (9). The fatty acid profile and particularly the balance between saturated fatty acids (SFA) and MUFA can be used as predictor for breast cancer (78-80). It was recently demonstrated that silencing of SCD1 in breast cancer cells does not affect cell viability but inhibits cell cycle progression (81). In these conditions, expressions of key proteins involved in cell cycle progression are decreased. The degree of SCD1 inhibition appears directly correlated with inhibition of cancer cells proliferation (19) decreasing the amount of SFA (SCD1 substrates), the main inhibitors of ACC (82).

Others studies point for a role of SCD1 in cancer progression and metastasis (Table II). It was shown that MUFA content in cholesterol esters is associated with higher death rate in cancer patients (83) while elevated levels of oleic acid were observed in breast cancers with metastasis (84), suggesting an increased activity of SCD1. Low content of stearic acid (SCD1 substrate) in phosphatidylcholine were also measured in breast tumors associated with subsequent metastasis (9). In breast adipose tissues, no difference in MUFA content was observed between benign tumors and normal tissues, but a

<table>
<thead>
<tr>
<th>Role</th>
<th>Type of cancer</th>
<th>System</th>
<th>Implicated mechanisms</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthesis of palmitate</td>
<td>Ovarian neoplasms</td>
<td>A2780 and SKOV3 cells</td>
<td>Oestrogen Akt-USP2a</td>
<td>(52, 60)</td>
</tr>
<tr>
<td></td>
<td>Breast carcinomas</td>
<td>MDA-MB-231, MCF-10A, BT474, and SKBr3 cells</td>
<td>EGF-oestrogen HER2/neu</td>
<td>(23, 48, 49, 51)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MCF7 cells</td>
<td>HER2/neu Lipid rafts</td>
<td>(62, 64)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SKBr3 cells</td>
<td>PI3K/Akt and ERK1/2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endometrial carcinomas</td>
<td>Patients</td>
<td>Oestrogen</td>
<td>(51)</td>
</tr>
<tr>
<td></td>
<td>Prostate cancers</td>
<td>Patients</td>
<td>Androgen</td>
<td>(50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mouse TRAMP iPrECs cells</td>
<td>Androgen</td>
<td>(53, 54)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LNCAp/ MDA-PCa-2a cells</td>
<td>Androgen</td>
<td>(55)</td>
</tr>
<tr>
<td></td>
<td>Renal cancers</td>
<td>Patients</td>
<td>HER2/neu-STAT3</td>
<td>(13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caki-1 cells Renca cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Head and neck neoplasms</td>
<td>Patients</td>
<td>EGFR</td>
<td>(71)</td>
</tr>
<tr>
<td></td>
<td>Lung carcinomas</td>
<td>Patients</td>
<td></td>
<td>(74)</td>
</tr>
<tr>
<td></td>
<td>Colon cancers</td>
<td>Mice xenograph models</td>
<td>MET-AKT-FAK</td>
<td>(76, 77)</td>
</tr>
<tr>
<td></td>
<td>Melanomas</td>
<td>Melanoma cell line B16-F10</td>
<td></td>
<td>(75)</td>
</tr>
<tr>
<td></td>
<td>Retinoblastomas</td>
<td>Patients</td>
<td></td>
<td>(69)</td>
</tr>
</tbody>
</table>
positive correlation was observed between MUFA concentration and metastasis (85). Alteration of SFA/MUFA ratio in breast tumors does not reflect the dietary intake of patients but rather the change in fatty acid metabolism in cells (86) underlying a role for de novo synthesized MUFA and SCD1.

In lung adenocarcinomas, SCD1 knockdown inhibits AKT phosphorylation and activity (20) known to be associated with cancer progression (87). Silencing SCD1 in SV40-transformed lung fibroblasts and in breast cancer cells inhibits GSK3β phosphorylation (20).

Consequently, nuclear β-catenin translocation is decreased leading to lower expression of cyclin D1 and vimentin, two proteins associated with a mesenchymal phenotype (88). Silencing SCD1 in MCF-7 and MDA-MB-231 breast cancer cells also increased E-cadherin expression associated with changes in cellular morphological aspects and decreased migration (81). It was also shown that palmitoleic acid (SCD1 product) is required to modify Wnt proteins leading to activation of the Wnt signaling pathway (89).

In breast cancer cells, we observed that the induction of β-catenin nuclear translocation by TGFβ is abrogated upon SCD1 silencing (Mounier et al., unpublished data). TGFβ acts as a tumor suppressor, but when cells become resistant to its action, it acts as a potent stimulator of malignant conversion (32). TGF-β activates SCD1 expression through a Smad-dependent pathway (88). Constitutive activation of the EGF signaling pathways through the ErbB receptors has been associated with metastasis and poor prognostic for patients (91). Paradoxically, incubation of breast cancer cells with oleic acid inhibits the expression of HER2/neu suggesting an anti-metastatic effect of the product of SCD1 (92).

The role of SCD1 in EMT probably involves GSK3β activation and downstream cellular events modifying cell adhesion and migration. Certain evidence also point for a role of TGFβ and EGF in mediating SCD1 expression in metastatic cancer cells.

6. Implication of other lipogenic genes

General modification of the lipid profile during cancer progression is associated with increased expression of several genes involved in lipid metabolism (84). Apart from FAS and SCD1, the expression of other genes was modulated such as ACC, INSIG1 (insulin-induced gene 1), SCAP (sterol regulatory element-binding protein cleavage-activating protein) and THRSP (thyroid hormone-responsive protein).

THRSP, also known as Spot14, is a nuclear protein that activates lipogenic genes (93). Low Spot14 expression was associated with prolonged survival in invasive breast cancers suggesting that Spot14 may not be a key player in EMT (93). Another study suggests that as breast cancer cells do not express lipoprotein lipase, lipids must be provided by a local environment such as breast lipids explaining why cancer cells with low Spot14 levels cannot survive in a low lipids concentration environment such as lymph nodes (94). The authors even suggest that elevated expression of Spot14 in cancer cells may provide a unique explanation for the elevated lipid synthesis in cancer cells.

Elevated ACC expression was also associated with a higher risk of infiltration in breast cancer (49). Amplification in ACC gene copy number was observed in breast cancer patients with reduced survival (95). Mutations in BCRA1, a gene associated with predisposition of inherited cancer, disrupt BCRA1 interaction with the inactive phosphorylated ACC. Consequently, ACC is dephosphorylated and activated (96). AMPK that phosphorylates ACC was also associated with prolonged survival in invasive breast cancers (97). In breast cancer cells, ACC is also regulated by a ubiquitin-dependent degradation process through its interaction with AKR1B10 (aldoketo reductase family 1 B10) (14). Pharmaceutical inhibition of ACC in cancer cells inhibits invadopodia formation, a membrane protrusion that facilitates matrix degradation and cellular invasion (98) but SCD1 is not required for invadopodia formation suggesting a different pathway. However, malonyl CoA decreases expression of the HER2/neu gene suggesting an anti-metastatic effect of ACC (99). This emphasizes the role of FAS in EMT as FAS decreases malonyl CoA content in cells.

Prostate cancer development and progression is often dependent of androgen and evidence indicates that androgen activation of the SREBP-dependent pathway may explain most of the androgen effects on lipogenesis (100). Androgen activates the cleavage of SREBP by increasing the

---

Table II. Role of SCD1 in metastatic cancers.

<table>
<thead>
<tr>
<th>Role</th>
<th>Type of cancer</th>
<th>System</th>
<th>Implicated mechanisms</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty acid desaturation</td>
<td>General</td>
<td>Patient</td>
<td></td>
<td>(83)</td>
</tr>
<tr>
<td>Formation of palmitolate and oleate</td>
<td>Breast cancers</td>
<td>MCF-7, MDA-MB-231, MDA-MB-435, MDA-MB-468, SKBR3, BT-474</td>
<td>ACC/AMPK, GSK3β/β-catenin</td>
<td>(9,78-80,83-86)</td>
</tr>
<tr>
<td></td>
<td>Lung cancer</td>
<td>SV40-W138, A549</td>
<td>PPARγ, AMPK/ACC, AKT/GSK3β</td>
<td>(18, 19, 20)</td>
</tr>
</tbody>
</table>
expression of SCAP and INSIG (57). SREBP1 also increases reactive oxygen species (ROS) production (101) inducing tumor suppression (102). A similar role of progesterone and EGF on SREBP cleavage and expression was reported in breast cancer cells (22,65,67).

7. Conclusions

Increased lipogenesis is an important hallmark of cancer progression and metastasis. Part of this effect is probably mediated by SREBP1. However, evidence points to a role of lipogenic enzymes independent of SREBP1. ACC activity is regulated in cancer cells through phosphorylation by AMPK or by interaction with BCRA1. However, malonyl CoA, the product of ACC, has an anti-metastatic effect suggesting a more direct role of FAS on EMT. As such, a role for palmitate in the formation of membranes and rafts was suggested. Rafts allow recruitment and stabilization of receptors such as EGF, HER2/ner and MET increasing cancer progression. In contrast to FAS, SCD1 is not involved in the formation of invadopodia suggesting that if both enzymes are involved in EMT, they probably act through different mechanisms. Increased SCD1 activity decreases the level of SFA, the main ACC inhibitors activating lipogenesis in cancer cells. SCD1 is also probably directly involved in EMT. FAS and SCD1 may be the most interesting targets for the treatment of metastatic cancers, and pharmaceutical inhibitors already exist that could be used readily for the treatment of patients.

References


