Increased lipogenesis in cancer

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Abbreviations: ATP, adenosine 5'-triphosphate; ACLY, ATP citrate lyase; ACC, acetyl CoA carboxylase; FASN, fatty acid synthase; RNAi, RNA interference; MAP kinase, mitogen-activated protein kinases; PI3K, phosphatidylinositol 3'-kinase; protein kinase B, AKT; AMPK, AMP-activated protein kinase; SREBP-1c, sterol regulatory element binding protein-1c

Cancer cells have adapted an altered metabolism to support their dysregulated proliferation. In human carcinoma, upregulation of several key enzymes involved in lipid synthesis is associated with tumor cell aggressiveness and a poor patient diagnosis. The phenomenon of elevated lipogenesis and lipogenic machinery in tumor cells has suggested lipogenic enzymes as potential diagnostic markers and therapeutic targets for cancer.

Introduction

Survival of multicellular organisms requires a tight control of cell proliferation. Genetic mutations in cancer cells alter receptor-initiated signaling pathways and lead to the constitutively active uptake and metabolism of nutrients that both promote cell survival and fuel cell growth. Altered metabolism of human cancer cells has been recognized since the 1920s, with Otto Warburg’s observation that cancer cells avidly consume glucose and produce lactic acid under aerobic conditions (the Warburg effect).1 However, why cancer cells rely on aerobic glycolysis has been a puzzle, since it is an inefficient way to generate adenosine 5'-triphosphate (ATP), the energy source for cells. Only until recently, it was recognized that metabolism in cancer cells is 'suspended' as circulating fatty acids (reviewed in refs. 2 and 5). Interestingly, the net conversion of glucose to lipids is dependent on the levels of extracellular lipids. Increased lipogenesis in cancer cells functions downstream from oncogenic signaling, and is linked to the well-known tumor-associated increase in glycolysis.2,5 It is believed that the high rate of de novo fatty acid synthesis in rapidly proliferating cells fuels membrane biogenesis and lipid-based protein modification.6,7 Knowledge of how lipid synthesis contributes to the proliferation needs for cancer cells will help to better understand the mechanistic links between cellular metabolism and growth control, and ultimately lead to better treatment of human cancer.

Increase of De novo Lipid Synthesis in Cancer Cells

In normal tissues and cells, even those with very high turnover, de novo fatty acid synthesis is 'suspended' as circulating fatty acids are sufficient and thus, are preferentially used for synthesis of cellular membrane and formation of signaling lipids. Consequently, lipogenic enzymes are expressed at low or undetectable levels in most human tissues and cells such as skeletal muscle, liver, mammary glands and epithelial cells (reviewed in ref. 5). In sharp contrast, tumor cells derive 95% of saturated and monosaturated fatty acids from de novo synthesis despite an abundant supply of extracellular fatty acids as evidenced in 14C glucose studies.8-10 This increased lipogenesis is reflected in a significantly elevated activity and expression of several key lipogenic enzymes in more recent studies, such as ATP citrate lyase (ACYL, the enzyme that generates cytosolic acetyl coenzyme A (CoA) from the cleavage of mitochondria-derived citrate), acetyl CoA carboxylase (ACC, which catalyzes the rate-limiting ATP-dependent carboxylation of acetyl CoA to malonyl CoA), fatty acid synthase (FASN, the main enzyme that catalyzes the condensation of acetyl CoA and malonyl CoA to produce long-chain fatty acids) (reviewed in refs. 2 and 5). Interestingly, the mRNAs of these enzymes are also coordinately overexpressed,11 supporting findings that they are regulated by similar signaling pathways.

The net conversion of glucose to lipids is dependent on the production of cytosolic acetyl-CoA from mitochondria-derived citrate through the action of ACLY. The chemical inhibitor SB-204990 and ACLY inhibition by RNA interference (RNAi) lead to a drastic reduction of glucose-dependent de novo lipid synthesis, and limits in vitro proliferation and survival of tumor cells in tumor cells displaying aerobic glycosis, such as human lung adenocarcinoma A549 cells,12,13 prostate PC3,12 and hematopoietic FL5.12 and K562 cells.14 The importance of lipogenesis for tumor growth and transformation is further supported by the fact that the inhibitor SB-204990 and small hairpin RNA (shRNA) knockdown of ACLY reduces tumorigenesis in nude mice.12,13

Besides inhibition of ACLY, RNAi knockdown of ACC, the rate-limiting enzyme, evokes a similar response in cancer cells.

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ACC overexpression has been shown at both the mRNA and protein levels in pre-neoplastic lesions and advanced carcinoma in human patients. It has been found that certain ACC sequence variants may associate with breast cancer susceptibility. Brusselmans and colleagues demonstrated that RNAi-mediated knockdown of ACC induced growth retardation and cell death via apoptosis in human prostate cancer cells. In the same study, the investigators determined that cell death occurred in the absence of cytosolic malonyl CoA accumulation, which is different from a mechanism observed after FASN inhibition. Similar to work done in prostate cancer cells, ACC activity is also essential for the survival of breast cancer cells. Specific knockdown of the ACC gene by RNAi in several breast cancer cell lines resulted in a collapse of mitochondrial membrane integrity and induction of apoptosis associated with depletion of palmitic acid.

Among all the lipogenic enzymes, FASN is most intensively studied. In fact, the implication of increased lipogenesis for cancer cell biology did not become a focus of interest until 1994, when Kuhajda and colleagues identified the oncogenic antigen-519 (OA-510), a marker of breast cancer in human patients with a markedly poorer prognosis, as FASN. Overexpression of FASN protein has been reported in many pre-neoplastic lesions and human epithelial cancers in breast, prostate, ovary, esophagus, stomach, lung, oral tongue, oral cavity, thyroid and endometrium, and also in mesothelioma, nephroblastoma, retinoblastoma, soft tissue sarcomas and Paget's disease of the vulva (reviewed in refs. 2 and 5). More importantly, clinical studies found an association between FASN and cancer progression. FASN overexpression and hyperactivity commonly connotes poor prognosis in breast cancer, prostate cancer, non-small cell lung cancer, malignant melanoma and soft-tissue sarcomas. Inhibition of FASN activity and expression by chemical and antisense approaches has been shown to be associated with suppression of cell growth and induction of apoptosis in several cancer cell lines.

The ultimate mechanisms responsible for increased lipogenesis in cancer cells may be at least partly attributed to the accumulation of the intermediate metabolite malonyl CoA, the fact that similar effects were observed when ACLY and ACC were targeted suggests that the fatty acid synthesis per se may be important for cancer cells. Moreover, deregulation of the fatty acid synthesis pathway often affects the synthesis of phospholipids in C14C-acetate labeling studies, suggesting that phospholipid synthesis accounts, at least in part (if not all), for the antitumor effects of inhibition of fatty acid synthesis. Indeed, some enzymes directly involved in the phospholipid synthesis have been reported to be important for the survival and proliferation of cancer cells. Choline kinase, the first enzyme involved in the synthesis of phosphatidylcholine, is significantly overexpressed in many cancers. Inhibition of choline kinase either induces cell death alone or increases the effects of other cancer therapeutic drugs.

**Some Key Upstream Signaling Pathways Regulating Lipogenesis in Cancer Cells**

The ultimate mechanisms responsible for increased lipogenesis are not completely understood yet. The upregulation of tumor-associated lipogenic enzymes can be regulated at both the transcriptional and posttranslational levels. Excessive growth factor signaling has emerged as a major contributor to the increased expression of multiple lipogenic enzymes at the transcriptional level, in particular, epidermal growth factor (EGF), keratinocyte growth factor (KGF) as well as the HER2 (erbB2/neu) receptor tyrosine kinase. The effects of growth factors and growth factor receptors are complex and involve activation and cross-talk between multiple signaling pathways such as mitogen-activated protein kinases (MAP kinases), phosphatidylinositol 3'-kinase (PI3K)/protein kinase B (AKT) pathway, and recently also AMP-activated protein kinase (AMPK), which in turn activate the lipogenic transcription factor, sterol regulatory element binding protein-1c (SREBP-1c).

In particular, the role of the PI3K/AKT pathway in controlling lipogenic enzyme expression has been well established. In the LNCaP prostate cancer cell line, pharmacological PI3K inhibitor, LY294002 or re-introduction of PTEN to PTEN-null LNCaP cells, markedly blunted FASN expression, activity and consequently fatty acid production. This effect could be reversed by a subsequent transfection of constitutively active AKT, thus suggesting that PI3K/AKT regulates FASN expression at the transcriptional level. Similar results have also been obtained in ovarian cancer cells. Induction of an exogenous AKT expression in an inducible stable cell line lead to an increase in fatty acids and phospholipids by upregulating the expression of genes involved in fatty acid biosynthesis, as a result of the synthesis and activation of nuclear SREBP-1c. Other studies have demonstrated similar findings on the correlation of FASN overexpression with the loss of PTEN and the subsequent activation of AKT. Finally, AKT may also directly interact with and regulate the activity of lipogenic enzymes. Several studies have demonstrated that AKT directly phosphorylates and activates ACLY, thereby enhancing lipogenesis and coupling glucose metabolism to lipogenesis.

In addition to the PI3K/AKT pathway, the MAP kinase pathway also regulates the expression of lipogenic enzymes through SREBP-1c. In both MCF7 and HCT human colon cancer cells, inhibitors of MAP kinase downregulated SREBP-1c expression while decreasing FASN promoter activity and thus FASN expression and fatty acid synthesis. H-ras transformation of MCF10A cells resulted in an upregulation of MAP kinase (as well as PI3K) signals with a concomitant increase in SREBP-1c levels, FASN expression and fatty acid synthesis. Deletion of...
the SREBP-1c response element from the FASN promoter significantly attenuated FASN promoter-dependent transcription as well as FASN protein expression and fatty acid synthesis in these cells.53

Fatty acid synthesis has also been linked to AMP-activated protein kinase (AMPK), a fuel-sensing enzyme that monitors changes in the AMP/ATP ratio regulating enzymes involved in fatty acid synthesis.65 Recently, it was demonstrated that acutely activated AMPK phosphorylates and inhibits ACC, while the same protein when chronically activated, decreases SREBP-1c, thus suppressing the synthesis of FAS, ACC and other lipogenic enzymes.56,66 Using activators of AMPK (5-aminooimidazole-4-carboxamide riboside or the thiazolidinedione rosiglitazone) several studies have recently demonstrated attenuated production of lipids, including phospholipids and cholesterol, as well as a marked attenuation in cell migration and proliferation in the S-phase of the cell cycle.55,67,68 These findings suggest that the fuel sensor and metabolic regulator AMPK is a potential target for treatment of cancer.

While several studies have demonstrated a correlation between elevated levels of FASN mRNA and protein,69-73 Rossi and colleagues have demonstrated a discrepancy in the correlation between FASN mRNA and protein levels in a small subset of prostate cancers in a gene array study.72 These results not only suggest that a non-transcriptional regulation of the enzyme exists, but also showed that in the population of cells with low- or undetectable levels of FASN protein and high FASN mRNA levels, pro-apoptotic genes such as tumor necrosis factor—alpha ligand and annexin V were upregulated. Regulation of FASN at the protein level is supported by the finding that FASN interacts with the ubiquitin-specific protease USP2a, which removes ubiquitin and stabilizes FASN in prostate cancer.72 Functional inactivation of USP2a results in decreased FASN protein and enhanced apoptosis.73

9. Oskhena M, Kannan R, Lyon J, Baker N, Liver and adipose tissue contributions to newly formed fatty acids in an acinar tumor. Am J Physiol 1984; 247:146-53.
10. Sabine JR, Abraham S, Chakoff IL. Control of lipid metabolism in hepatomas: sensitivity of rate of fatty acid and cholesterol synthesis by mouse hepatoma BW7576 to fasting and to feedback control. Cancer Res 1967; 27:793-9.
11. Yahagi N, Shimano H, Hasegawa K, Ohashi K, Matsumoto T, Naima Y, et al. Co-ordinate activation of lipogenic enzymes in hepatocellular carcinoma. Eur J Cancer 2005; 41:1316-22.
12. Haritatsiluou G, Zhao F, Bauer DE, Andreadis C, Shaw AN, Bhanak D, et al. ATP citrate lyase inhibition can suppress tumor cell growth. Cancer Cell 2005; 8:311-21.
13. Migita T, Narita T, Nomura K, Miyagi E, Inazuka F, Matsuoka M, et al. ATP citrate lyase activation and therapeutic implications in non-small cell lung cancer. Cancer Res 2008; 68:8547-54.
14. Bauer DE, Haritatsiluou G, Zhao F, Andreadis C, Thompson CB. ATP citrate lyase is an important component of cell growth and transformation. Oncogene 2005; 24:6314-22.
15. Milgrau LZ, Winters LA, Pasternack GR. Kuhajda FP. Enzymes of the fatty acid synthesis pathway are highly expressed in in situ breast carcinoma. Clin Cancer Res 1997; 3:2151-20.
16. Sinilnikova OM, Ginolhac SM, Magnaud C, Leone M, Anczukow O, Hughes D, et al. Acetyl-CoA carboxylase alpha gene and breast cancer susceptibility. Carcinogenesis 2004; 25:2417-24.
17. Sinilnikova OM, McKay JD, Tartaglia SV, Czanit F, DeSilva D, Bissy C, et al. Haploype-based analysis of common variation in the acetyl-coa carboxylase alpha gene and breast cancer risk: a case-control study nested within the European Prospective Investigation into Cancer and Nutrition. Cancer Epidemiol Biomarkers Prev 2007; 16:409-15.
18. Brusselmans K, De Schrijver E, Verhoeven G, Swinnen JV. RNA interference-mediated silencing of the acetyl-CoA carboxylase alpha gene in human breast cancer cells confers cancer cells with a profound growth and/or survival advantage. Cancer Res 2003; 63:6765-70.
19. Hennigar RA, Jenner K, Wood FD, Kuhajda FP. Potential mediator of cytotoxicity induced by fatty acid synthesis inhibition in human breast cancer cells and xenografts. Cancer Res 2000; 60:213-8.
20. Zhou W, Simpson PJ, McFadden JM, Townsend CA, Medghalchi SM, Vaidlamudi A, et al. Fatty acid synthesis inhibition triggers apoptosis in phase S in human cancer cells. Cancer Res 2003; 63:7310-7.
21. Chajes V, Gambor M, Moreau K, Lenior GM, Joulton V. Acetyl-CoA carboxylase alpha is essential to breast cancer cell survival. Cancer Res 2006; 66:5287-94.
22. Kuhajda FP, Jenner K, Wood FD, Hennigar RA, Jacobs LB, Dick JD, et al. Fatty acid synthesis: a potential selective target for antineoplastic therapy. Proc Natl Acad Sci USA 1994; 91:6379-83.

Conclusion
The metabolism of cancer cells is adapted to facilitate the uptake and incorporation of nutrients into biomass, e.g., nucleotides, amino acids and lipids.3 The concept that exacerbated lipogenesis confers cancer cells with a profound growth and/or survival advantages over those that maintain physiological levels of lipid biosynthesis strongly suggests that some lipogenic enzymes might work as metabolic intermediates of oncogenesis.2 In deed, inhibition of several lipogenic enzymes by RNAi and small inhibitors impairs cancer cell proliferation and evokes a decrease in the viability of cancer cells (reviewed in refs. 2 and 5). Interestingly, no cytotoxic effects of ACC and FASN inhibition were observed in normal human mammary epithelial cells even though de novo fatty acid synthesis did decline in these cells.31 This finding suggests that survival of normal differentiated cells may synthesize lipids primarily using fatty acids taken from dietary or culture medium and are less dependent on the de novo fatty acid synthesis, whereas cancer cells are highly addicted to the de novo fatty acid synthesis. The differential requirement of the de novo fatty acid synthesis in normal and cancer cells supports the potential success of targeting the lipogenic pathway for cancer therapy.

Cancer-associated metabolic changes have come to be a hot topic in cancer research in recent years. Many questions still remain to be explored. Understanding the regulation and signaling regulatory network of metabolic adaption of lipogenesis in cancer cells will certainly lead to improved cancer therapeutic strategies.

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Iorio E, Mezzanzanica D, Alberti P, Spadaro F, Lupu R, Menendez JA. Pharmacological inhibitors of fatty acid synthase inhibit chemopreventive monotumoriastic human mammary epithelial cells and breast tumor progression. Cancer Res 2005; 65:11034-43.

Ramirez de Molina A, Banex-Comelon M, Guitierrez R, Rodriguez-Gonzalez A, Olmeda D, Megias D, et al. Choline kinase activation is a critical requirement for the proliferation of primary human mammary epithelial cells and breast tumor progression. Cancer Res 2004; 64:6372-9.

Rodriguez-Gonzalez A, Ramirez de Molina A, Banex-Comelon M, Megias D, Lacal JC. Inhibition of choline kinase renders a highly selective cytotoxic effect in tumour cells through a mitochondrial independent mechanism. Oncogene 2005; 24:6169-78.

Mori N, Glande K, Takagi T, Raman V, Bhuwana ZM. Choline kinase downregulation increases the effect of 5-fluorouracil in breast cancer. Cancer Res 2007; 67:11284-90.

Swinnen JY, Heyns W, Deboel L, Foulfeille F, Heyns W, Verhoeven G. Stimulation of tumor-associated fatty acid synthase expression by growth factor activation of the steroid regulatory element binding protein pathway. Oncogene 2000; 19:5173-81.

Chang Y, Wang J, Liu X, Thewke DP, Mason RJ. KGF activates a MAPK/RNA polymerase II kinase renders a highly selective cytotoxic effect in tumor cells through a mitochondrial independent mechanism. J Biol Chem 2003; 278:33895-900.

Furuta E, Pai SK, Zhan R, Bandopadhyay S, Watabe M, Mo YY, et al. Fatty acid synthase gene is upregulated by hypoxia via activation of Akt and sterol regulatory element binding protein-1. Cancer Res 2008; 68:1003-11.

Sundqvist A, Bengoechea-Alonso MT, Ye X, Lukijanchuk V, Jin J, Harper JW, et al. Control of lipid metabolism by phosphorylation-dependent degradation of the SREBP family of transcription factors by SCD (Fw7). Cell Metab 2005; 1:379-91.

Van de Sande T, De Schrijver E, Heyns W, Verhoeven G, Swinnen JV. Role of the phosphatidylinositol 3-kinase/Akt kinase pathway in the overexpression of fatty acid synthase in LNCaP prostate cancer cells. Cancer Res 2002; 62:642-6.

Perzmann T, Griffiths B, Chuang YL, Delpuech O, Griffiths JR, Dowward J, et al. PKB/Akt inducible transcription of enzymes involved in cholesterol and fatty acid biosynthesis via activation of SREBP. Oncogene 2005; 24:10089-1008.

Sundberg F, Mohandas T, Jemal A, Wright J, et al. Upregulation of acetyl-CoA carboxylase alpha and fatty acid synthase by human epidermal growth factor receptor 2 at the translational level in breast cancer cells. J Biol Chem 2007; 282:26122-31.

van de Sande T, De Schrijver E, Heyns W, Verhoeven G, Swinnen JV. Role of the phosphatidylinositol 3-kinase/Akt kinase pathway in the overexpression of fatty acid synthase in LNCaP prostate cancer cells. Cancer Res 2002; 62:642-6.

Perzmann T, Griffiths B, Chuang YL, Delpuech O, Griffiths JR, Dowward J, et al. PKB/Akt inducible transcription of enzymes involved in cholesterol and fatty acid biosynthesis via activation of SREBP. Oncogene 2005; 24:10089-1008.

Sundberg F, Mohandas T, Jemal A, Wright J, et al. Upregulation of acetyl-CoA carboxylase alpha and fatty acid synthase by human epidermal growth factor receptor 2 at the translational level in breast cancer cells. J Biol Chem 2007; 282:26122-31.

Van de Sande T, De Schrijver E, Heyns W, Verhoeven G, Swinnen JV. Role of the phosphatidylinositol 3-kinase/Akt kinase pathway in the overexpression of fatty acid synthase in LNCaP prostate cancer cells. Cancer Res 2002; 62:642-6.

Furuta E, Pai SK, Zhan R, Bandopadhyay S, Watabe M, Mo YY, et al. Fatty acid synthase gene is upregulated by hypoxia via activation of Akt and sterol regulatory element binding protein-1. Cancer Res 2008; 68:1003-11.

Sundqvist A, Bengoechea-Alonso MT, Ye X, Lukijanchuk V, Jin J, Harper JW, et al. Control of lipid metabolism by phosphorylation-dependent degradation of the SREBP family of transcription factors by SCD (Fw7). Cell Metab 2005; 1:379-91.

Yoon S, Lee MY, Park SW, Moon JS, Koh YK, Ahn YH, et al. Upregulation of acetyl-CoA carboxylase alpha and fatty acid synthase by human epidermal growth factor receptor 2 at the translational level in breast cancer cells. J Biol Chem 2007; 282:26122-31.

van de Sande T, De Schrijver E, Heyns W, Verhoeven G, Swinnen JV. Role of the phosphatidylinositol 3-kinase/Akt kinase pathway in the overexpression of fatty acid synthase in LNCaP prostate cancer cells. Cancer Res 2002; 62:642-6.