The role of USF1 in the regulation of lipogenesis and breast cancer tumor progression

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Abstract

Breast cancer accounts for nearly one-quarter of all cancer diagnoses and is the principal cause of cancer-related mortality in women worldwide. Triple negative breast cancer (TNBC) is a clinically aggressive subtype of breast cancer commonly resistant to therapeutics that have been successful in increasing survival in patients with ER+, PR+ and HER2+ breast cancer subtypes. As such, identifying factors that contribute to poor patient outcomes and mediate the growth and survival of TNBC cells remain important areas of investigation. USF1 (upstream stimulatory factor 1), a gene linked to drive lipogenesis and cellular proliferation, is overexpressed in human malignancies, yet its contribution to cancer remains unclear. In analyzing large number of genomic datasets including The Cancer Genome Atlas (TCGA), we found that USF1 expression is significantly higher in TNBC patient samples. Also, USF1 gene expression positively correlates with key lipogenic enzymes. Significantly, we found that high expression of USF1 in breast cancer correlates with decreased patient survival. We therefore hypothesize that USF1 promotes breast tumorigenesis and progression by activating lipogenic gene expression. We conducted pilot in vitro studies to determine the influence of USF1 expression and cell proliferation. It was demonstrated that knockdown of USF1 decreased cellular proliferation in 2D cell culture of the TNBC cell line MDA-MB-231. We also assessed the effects of USF1 expression levels on in vivo tumor growth. We found that USF1 overexpression and knockdown enhanced and reduced tumor growth in vivo, respectively. In global lipid analysis, USF1 overexpression correlated with increased lipid production. Further studies are underway to determine the mechanisms by which USF1 promotes tumorigenesis and metastatic progression. Our studies will shed lights on roles of USF1 in breast cancer tumor biology.

Introduction

Lipids play important roles during tumor initiation and disease progression. Tumors undergo exacerbated de novo lipogenesis described as a significant increase in fatty acids, and lipogenic enzymes such as sterol regulatory element-binding protein (SREBP) and fatty acid synthase (*FAS*).[1] USF1 is a basic-Helix-Loop-Helix-Leucine Zipper transcription factor that regulates the transcription of *FAS* and SREBP1c.[2] Previous studies reported high expression of USF1 in basal-like tumors such as TNBC.

TNBC is an aggressive and metastatic subtype of breast cancer that causes poor patient survival and life-threatening comorbidities. Currently, there are limited treatments and prognostic markers for the assessment of this disease. Understanding how lipogenesis induces tumorigenesis especially in aggressive tumors has been an emerging issue to evaluate new molecular targets for drug discovery.

Hypothesis and Aims

USF1 promotes breast tumorigenesis and progression by activating lipogenic gene expression

Aim 1: Determine roles of USF1 in tumorigenesis and metastasis using TNBC xenograft models and in-vitro assays

Aim 2: Investigate molecular mechanism by which USF1 promotes tumorigenesis

Materials and Methods

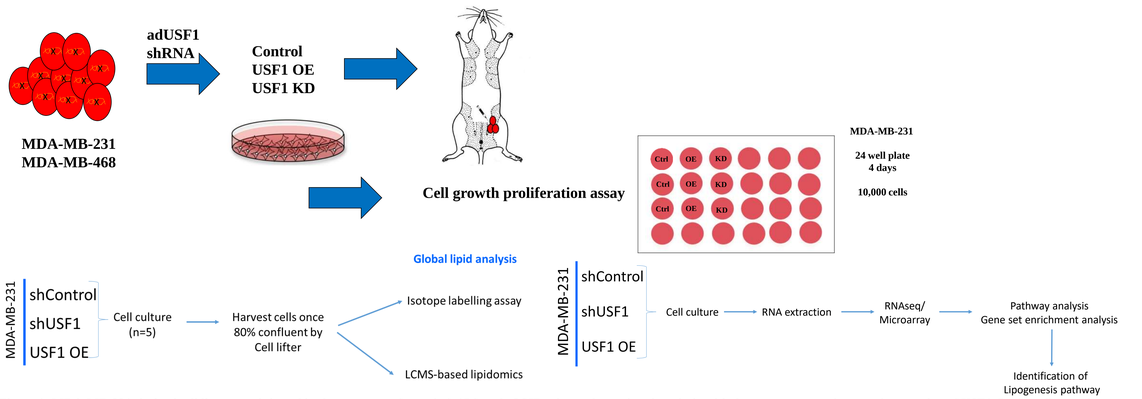

Figure 1: MDA-MB-231-derived cell lines were injected in the mammary fat pad of 15 female SCID mice to determine the relationship between tumor volume and expression of USF1. In the cell growth proliferation assay, cell number was recorded for each sample: control, USF1 overexpression and knockdown for four days. For global lipid analysis, MDA-MB-231-derived cell lines (control, shUSF1 and USF1 OE) will be used to measure the difference in lipid production. RNAseq will be performed using MDA-MB-231-derived cell lines grown in either serum-free or complete medium to identify molecular targets in the lipogenesis pathway.

Results

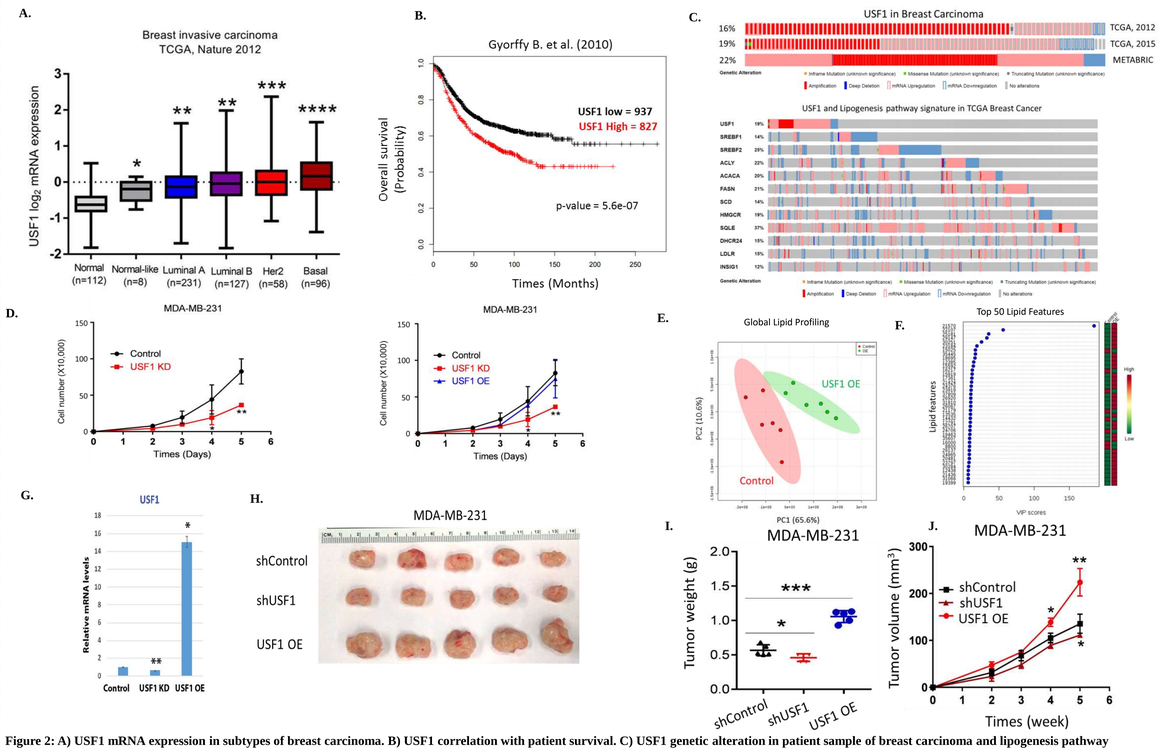

Figure 2: A) USF1 mRNA expression in subtypes of breast carcinoma. B) USF1 correlation with patient survival. C) USF1 genetic alteration in patient sample of breast carcinoma and lipogenesis pathway signature . D) Cell growth proliferation assay showing the difference in cell number for each sample. E) Global lipid analysis of USF1 overexpression in MDA-MB-231 cells. F) The overexpression of USF1 caused an increase in lipid production. G) Validation of USF1 knockdown and overexpression. MDA-MB-231 cells with a control, a USF1 shRNA and the USF1 cDNA vector were subjected to qRT-PCR. USF1 RNA levels were normalized against ACTB levels. *: p<0.05, **: p<0.01 (t-test). H) In the NSG/SCID model, the tumor size is compared between each group of mice. The tumor size is increased when there is a higher expression of USF1. I) The overexpression of USF1 (USF1 OE) has a higher tumor weight than the shControl and shUSF1 (USF1 knockdown) groups. J) On the fifth week, the tumor in the USF1 overexpression group has a larger volume when compared to shControl, p<0.01. While the shUSF1 has the smallest tumor volume. n=5, *p<0.05, **p<0.01,*p<0.001**

Conclusions

It is known that high expression of USF1 is present in TNBC tumor samples and cell lines. As a result, its upregulation leads to poor patient survival in this disease. Genetic alteration analysis in breast carcinoma indicate that USF1 amplification is a relevant component in many patient cases. In the growth proliferation assay, there is a significant difference between the control and USF1 knockdown. The reduction in the expression of USF1 hinders proliferation that is usually representative of aggressive phenotype in cancer. There is an increase in tumor weight and volume when USF1 is overexpressed in the MDA-MB-231 cells. The USF1 knockdown group has the smallest tumor volume and weight which signifies that USF1 has a major role in inducing tumor growth and promoting the metastatic potential of breast cancer cells. The overexpression of USF1 has distinct lipid profile and causes an increase in lipid production that influences the occurrence of metastasis in breast cancer. We hope to identify molecular targets in the lipogenesis pathway via RNAseq to understand how USF1 promote tumorigenesis in breast cancer. Understanding how USF1 interacts with regulators of lipogenesis such as FASN and SREBPs will establish its role in the lipogenesis pathway and elucidate a novel functionality of USF1.

TNBC is a highly metastatic subtype of breast cancer, resistant to common therapies that have been successful in patients diagnosed with other subtypes. Identifying molecular targets for drug discovery and clinical biomarkers to evaluate patient prognosis are important areas of investigation to improve the management of this disease.

Future Works

- **Determine if USF1 overexpression promotes metastasis.**
- **Elucidate the mechanism by which USF1 promotes de novo lipogenesis, tumorigenesis and metastatic progression.**
- **Determine the correlation between USF1 expression and lipid production via mass spectrometry.**

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