FASN inhibition as a potential treatment for therapy of endocrine resistant breast cancer

Aleksandra Gruslova [1], Gangadhara Sareddy [2], Ratna Vadlamudi [2], Suryavathi Viswanadhapalli [2], Virginia Kaklamani[1], Kate Lathrop[1], Andrew Brenner[1]

[1] Mays Cancer Center at UT Health San Antonio MD Anderson, TX; [2] University of Texas Health at San Antonio, TX

Abstract

INTRODUCTION: Fatty acid synthase (FASN) is a key enzyme in tumor cell biology controlling endogenous lipid biosynthesis. It is overexpressed in a biologically aggressive subset of tumors, including breast carcinoma. We previously reported prolonged stabilization of disease with TVB-2640 in patients with advanced metastatic breast cancer, including some endocrine resistant ER+ tumors. Using in vitro and in vivo models, we assessed the role of FASN inhibition by TVB-3166 (preclinical version of TVB-2640) for treatment of endocrine resistant breast cancer. **METHODS:** Breast tumor cells were incubated with TVB-3166 (200nM), imaged and analyzed by automated Live-Cell analysis system (IncuCyte). For tumor growth inhibition, cells ($2X10^6$) were subcutaneously injected into SCID mice implanted with estrogen pellets. Once tumors were measurable, mice were divided into treatment groups: tamoxifen (4mg/kg), TVB-3166 (60mg/kg) and the combination. Patient tumor explants were incubated for 72h on gelatin sponges in culture medium in the absence or presence of 200nM TVB-3166. Tissue were fixed in 10% formalin and processed into paraffin blocks. Sections were stained with H&E, ERα and Ki67. **RESULTS:** The effectiveness of FASN inhibition on the growth of tumor cells has been confirmed in a number of breast cancer cell lines such as MCF7, ZR75, MDA-MB-231 and others. TVB-3166 leads to a marked inhibition of growth in tamoxifen resistant (TamR) cells, which 15% greater than in the parent line. IHC and Western blot showed FASN inhibition leads to significantly reduction of ERα levels. Immunofluorescent confocal microscopy showed inhibition of FASN by TVB-3166 alters subcellular localization of ERα. TVB-3166 was able to significantly inhibit tamoxifen resistant breast tumor growth in mice ($p<0.05$). Additionally, TVB-3166 treatment of primary tumor explants decreased their proliferation (Ki67) compared to untreated controls (14% vs 36%, $p<0.01$). **CONCLUSION:** Our preclinical data provide evidence that FASN inhibition by TVB-3166 presents a promising therapeutic strategy for treating of endocrine resistant breast cancer. RNA sequencing of tumor explants is being performed to evaluate FASN inhibition impact on canonical and non-canonical ERα signaling pathways.

Materials and Methods

For in vitro drug screening, breast tumor cells were plated in 24-well plates in growth medium (DMEM containing 10% FBS and 1% L-glutamine), 24-h after cell plating, medium was replaced with Advanced MEM containing 1% charcoal-stripped FBS, 1% L-glutamine. TVB_3166 was added to each treatment well (200nM). Plates were incubated, imaged and analyzed by automated Live-Cell analysis system (IncuCyte). For breast tumor growth in vivo, MCF7 tamoxifen resistan cells ($2X10^6$) were subcutaneously injected into SCID mice implanted with estrogen pellets. Once tumors were measurable, mice were divided into 3 treatment groups: tamoxifen (4mg/kg), TVB-3166 (60mg/kg) and the combination. IHC analysis of ER performed on xenograft tumors. Patient tumor explants were incubated for 72h on gelatin sponges in culture medium in the absence or presence of 200nM TVB-3166. Tissue were fixed in 10% formalin and processed into paraffin blocks. Sections were stained with H&E, ERα and Ki67. NGS libraries were prepared from total RNA following Illumina TruSeq stranded RNA Sample Prep kit and sequenced with 50bp single-read module using Illumina HiSeq 3000 system. After adapter trimming and demultiplexing, short-read sequence reads were processed using TopHat2 and HTSeq for genome alignment and expression quantification.

Results

Tamoxifen resistant (TamR) cells showed greater sensitivity to FASN inhibition than sensitive parental lines. The effectiveness of FASN inhibition on the growth of tumor cells has been confirmed in a number of breast cancer cell lines such as MCF7, ZR75, MDA-MB-231 and others. TVB-3166 has increased activity against TamR ER-driven breast cancer cell lines with 60% decrease in growth rate (Figure 1B) relative to parental with a 40% decrease in growth (Figure 1A). Further, the addition of TVB-3166 led to near complete growth inhibition in both Tam sensitive and resistant lines.

FASN inhibition leads to significant reduction of ERα levels. Western blot analysis of ERα showed a time dependent loss of protein expression in MCF7 which was markedly greater in TamR cells (Figure 2A). Following 72h of exposure protein levels decreased by 90% in the TamR sells, while only 20% in the TamS cells. Immunofluorescent confocal microscopy indicated that TVB-3166 alters subcellular localization of ERα (HOW????),

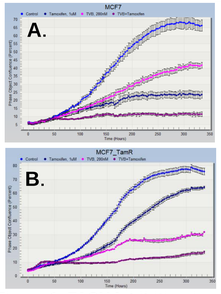


Figure 1. FASN Inhibition in TamS (A) or TamR (B) MCF7

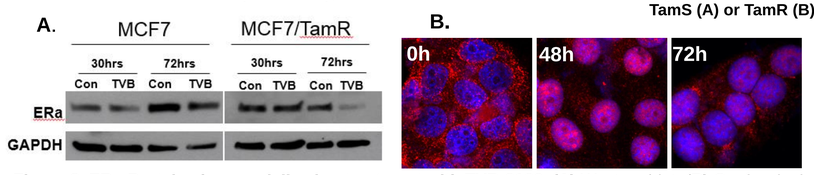
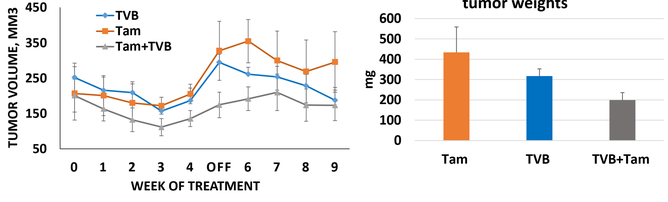


Figure 2. ERα Protein changes following treatment with TVB-3166. **(A)** Western blot. **(B)** Confocal microscopy.

TVB-3166 was able to significantly inhibit tamoxifen resistant breast tumor growth in mice. 8 weeks of treatment with TVB alone or in combination with Tamoxifen resulted in statistically significant inhibition of MCF7 tamoxifen resistant xenografts growth (n=6 mice/group) compared to tamoxifen alone. Animals tolerated treatment very well without effect on weight. These data indicate that TVB-3166 is a potent inhibitor of the growth of endocrine resistant breast tumors in vivo with no overt signs of toxicity in mice.

Figure 3. TVB-3166 inhibited MCF7 TamR xenograft growth.
(A) Tumor growth curve during the treatment.
(B) Extirpated tumor weights at the end of the treatment.



TVB-3166 inhibits proliferation and leads to loss of ERα protein in patient derived tumor explants

IHC analysis of TVB treated tumors showed significant decrease of ER levels after TVB treatment alone or in combination with tamoxifen ($p=0.002$ and $p=0.003$, respectively).

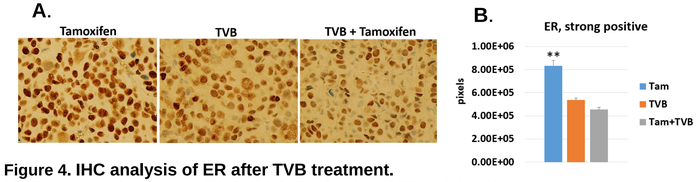


Figure 4. IHC analysis of ER after TVB treatment.
(A) Representative images **(40x). (B)** Quantification of ER staining (Aperio).

In addition, TVB-3166 treatment of primary breast tumor explants reduced their proliferation (Ki67 staining) compared to untreated explants (14% vs 36%, $p<0.001$), and also led to significant reduction the ER levels in tumors ($p<0.01$).

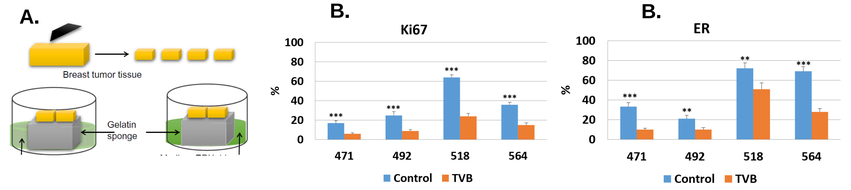


Figure 5. TVB treatment of patient-derived explants. (A) Schematic representation of ex vivo culture model. Quantification of IHC staining of tissue with Ki67 **(B)** and ER **(C)**.

4. TVB-3166 interacts with ER-driven breast cancer signaling pathways

RNA sequencing analyses revealed that TVB altered the expression of 309 genes ($p<0.01$) in primary derived breast tumors compare to vehicle control. Using fold-change to rank order genes, also were performed gene-set enrichment analysis (GSEA) and 2 of enriched gene sets: genes down-regulated in ESR1 positive breast tumors compared to the ESR1 negative tumors; and Invasiveness signature resulting from cancer cell-microenvironment interaction.

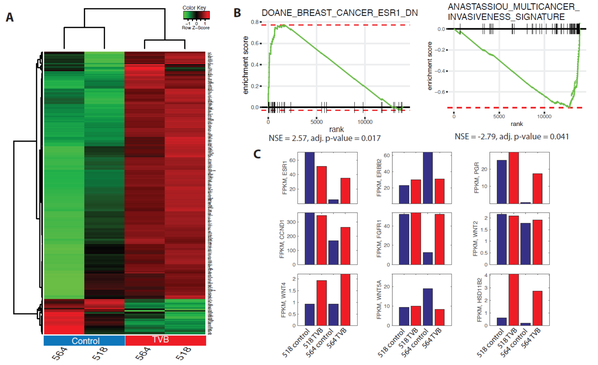


Figure 6. RNA-seq analyses of primary tumor explants after 72h of TVB treatment. (A) The heat map of differentially expressed genes between vehicle and TVB. **(B)** GSEA testing correlation between TVB regulated and 2 of enriched gene sets. **(C)** bar graph for several candidate genes expression (blue -control, red - TVB).

Conclusions

Our preclinical data provide evidence that FASN inhibition by TVB-3166 presents a promising therapeutic strategy for treating of endocrine resistant breast cancer.

Funding and Acknowledgment

This study was funded by a pilot grant through the Mays Cancer Center, NIH P30CA054174

We thank our patients for providing their tumors for research and UT Health Genome Sequencing Facility (GCCRI, San Antonio) and Dr. Yidong Chen for generation of genomic data and providing support with its analysis.