DIO3, the Thyroid Hormone Inactivating Enzyme, Promotes Tumorigenesis and Metabolic Reprogramming in High Grade Serous Ovarian Cancer

MEIR MEDICAL CENTER
Affiliated with the Sackler Faculty of Medicine, Tel Aviv University

Dotan Moskovich [1,2], Adi Alfandari [1,2], Yael Finkelshtein [1,2], Avivit Weisz [4], Aviva Katzav [4], Debora Kidron [3,4], Evgeny Edelstein [4], Daniel Veroslavski [5], Ruth Perets [5], Nissim Arbib [3,6], Yfat Kadan [6], Ami Fishman [3,6], Bernard Lerer [7], Martin Ellis [1,3] and Osnat Ashur-Fabian [1,2]

[1]Translational Oncology Laboratory, Meir Medical Center [2] Human Molecular Genetics and Biochemistry, [3] Sackler Faculty of Medicine, Tel Aviv University [4] Pathology Dept. Meir Medical Center [5] Clinical Research Institute at Rambam [6] Obstetrics and Gynecology, Meir Medical Center [7] Biological Psychiatry Laboratory Hadassah

INTRODUCTION

- High grade serous ovarian carcinoma (HGSOC) is the most lethal gynecologic malignancy. The source of HGSOC is in PAX-8 positive secretory cells of the fallopian tube (FT).
- The thyroid hormone, T3, is considered a tumor suppressor by promoting cell differentiation and mitochondrial respiration.
- Tumors evolved a strategy to avoid T3 anticancer actions by expressing its catabolizing enzyme, Deiodinase type 3 (DIO3). This stimulates cancer proliferation and aerobic glycolysis (Warburg effect).
- DIO3 is highly expressed during fetal development, less frequent in most healthy adult tissues and is reexpressed in cancer tissues

OBJECTIVES

Our research aims:

- Study DIO3 expression in HGSOC cells and human tissues
- Study the role of DIO3 in HGSOC progression

METHOD

- HGSOC, ovary, FT, DIO3 knockdown (DIO3-KD) (shRNA) cells
- Western blot (WB): DIO3/other proteins
- Immunohistochemistry (IHC) for tissues

METHOD (Continued)

- Soft agar colony assay
- Flow cytometry (Annexin-PI)
- Proteomics analysis
- Animal studies: mice were inoculated with control (left flank) or DIO3-KD cells (right flank) and followed for tumor growth for 28 days

RESULTS

DIO3 was expressed in HGSOC cells (**Fig. 1A**). While absent in normal ovaries, DIO3 was expressed in secretory cells of normal fallopian tube (FT) and premalignant serous tubular in situ carcinoma (STIC) lesion, considered the disease site-of-origin and tumor tissues from patients (**Fig.1B**). DIO3-KD (**Fig. 2A**) led to attenuated cell growth and accumulation of apoptotic cells aggregates (**Fig. 2B**), which were confirmed by Annexin-PI (**Fig. 2C**), reduced colony formation (**Fig. 2D**) and altered oncogenes and metabolic-related proteins (**Fig. 3**).

Proteomics analysis further indicated that DIO3 silencing altered an array of cancer-relevant proteins, the majority of which are involved in metabolic reprogramming (**Fig. 4**). DIO3-KD inoculated into nude mice resulted in significant tumor growth inhibition compared to control cells (**Fig 5**).

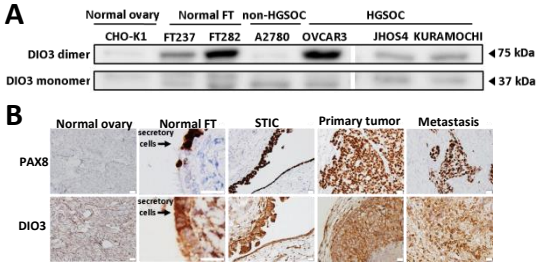

Fig. 1. DIO3 expression (A) WB of ovary, FT and HGSOC cells **(B)** IHC analysis for normal ovary and FT's, STIC, tumor and colon metastasis of human HGSOC tissues

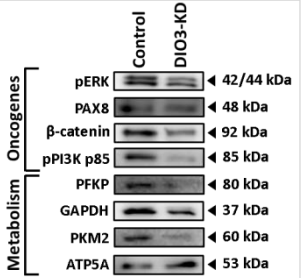

Fig. 3. Oncogenes/metabolic proteins (WB)

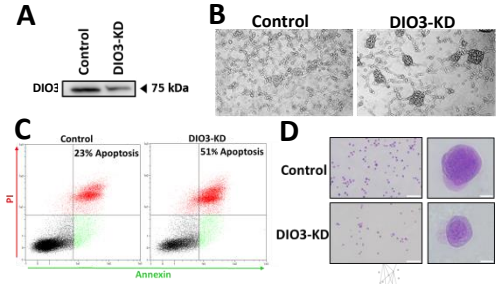

Fig. 2. DIO3 silencing (A) WB **(B)** Microscopy **(C)** Apoptosis **(D)** Colony formation

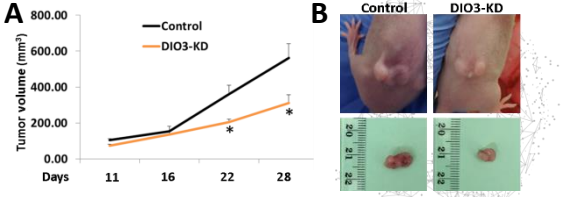

Fig. 5. DIO3 inhibits tumor growth in mice (A) Tumor volume **(B)** Images and size

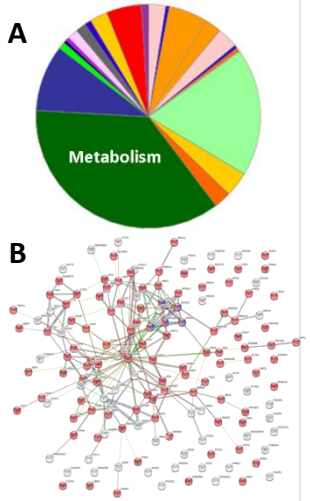

Fig. 4. Proteomics (A) PANTHER (B) Interaction network (STRING)

CONCLUSIONS

To conclude, our collective data establish the involvement of DIO3 in HGSOC progression and proposes this enzyme as a promising target for inhibition