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Identification of Novel Ovarian Cancer Oncogenes that Function by Regulating Exosome Function

TITLE: Identification of novel ovarian cancer oncogenes that act by regulating exosome function

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13. SUPPLEMENTARY NOTES

14. ABSTRACT : The first year of this award included the following tasks
Major Task 1: ACURO approval of animal studies - accomplished
Major Task 2: HRPO approval of human studies - accomplished
Major Task 3: Generation of cells expressing Fluc/Gluc and different levels of SYTL2/Rab-GTPase genes_ generated multiple cells expressing Flic/Gluc and SYTL2
Major Task 4: In vitro behavior of cells from task 3 - accomplished
Major Task 5: In vivo behavior of cells from task 3 - accomplished

15. SUBJECT TERMS
Ovarian Cancer, oncogenes, SYTL2, exosomes

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Introduction

Epithelial ovarian cancer affects 21,000 women resulting in approximately 14,000 deaths in the US per year. Even though, ovarian cancer has >75% response rate to platinum and taxane chemotherapy following debulking surgery, most women will develop recurrent tumors which become resistant to conventional therapeutics. A critical need to improve the management of ovarian cancer is identifying drivers of this tumor that elucidate the underlying biology and ultimately the clinical behavior of the disease. This will lead to the development of novel targeted therapies for platinum-resistant cancers.

Through careful analysis of the amplicons found in ovarian cancer we have published the identification of genes that induce tumor progression through novel mechanisms of action and are potential therapeutic targets. We have now identified another gene, Synaptotagmin-like 2 (SYTL2) as a potential driver gene of ovarian cancers. SYTL2, located in a major amplicon (11q13-11q14), was found to be amplified in 25% of ovarian cancer patients and is associated with significantly poorer survival. Even though SYTL2 remains a largely uncharacterized protein, previously published data indicate its involvement in melanosome vesicle based protein secretion and cell-cell communication through interaction with Rab27a or Rab27b. Rab27 belongs to the Rab superfamily of GTPases which includes multiple members that are known to participate in exosome release and to be associated with tumor progression.

Exosomes are 30-100 nm membrane-bound vesicles secreted by various types of cells. Numerous cancer studies showed that solid tumours release large quantities of exosomes transporting oncoproteins and immune suppressive molecules in order to support tumor development, metastasis and resistance to therapy. Studies of ovarian and endometrial human tumors characterizing the contents of circulating exosomes revealed a potential role for these particles in inducing tumor dissemination and angiogenesis as well as suppressing the immune system against cancer development and progression.

We hypothesize that SYTL2 and members of the Rab superfamily of GTPases represent a new class of oncogenes that promote tumor progression by regulating exosome secretion and modulating communication between cancer cells and their environment. The goal of this project is to investigate whether SYTL2 and members of the Rab superfamily regulate the release of exosomes and analyze how this modulates the biology of ovarian cancer cells and the tumor's clinical features.

Our hypothesis is supported by recent preliminary data indicating that overexpression of SYTL2 in a human ovarian cancer cell line increases proliferation, migration/invasion and cisplatin-resistance of these cells, thus highlighting the tumorigenic role of this gene. SYTL2 was also shown to induce secretion of exosomes from these ovarian cancer cells. Deletion of the Rab27 binding domain of the SYTL2 gene suppressed its capacity to release exosomes as well as to induce migration and invasion of ovarian cancer cells, thus further supporting a functional link between SYTL2 and the Rab superfamily of GTPases. In addition, through meta-analysis of 15 published data sets for gene expression, amplification, and association with poor patient prognosis, we have identified at least other two members of the Rab superfamily that are potential drivers of ovarian cancer (Rab2b and Rab31).

KEYWORDS: Ovarian Cancer, genomic profiling, oncogenes, exosomes, SYTL2

Research Accomplishments

Major Tasks 1 and 2: Obtain ACURO and HRPO approval. Months 1-3

We obtained both ACURO and HRPO approval for the studies described in this award.

Major Task 2: To generate OC cell lines expressing Flcuc/Gluc and in which expression of SYTL2 is modified. We have generated 3 ovarian cancer cells (SKOV3, OVCAR5, and CAOV3) to express Fluc/Gluc. In SKOV3 and CAOV3 cells, which had deleted endogenous SYTL2, we have induced ectopic over-expression of SYTL2 through transfection with an SYTL2 expressing plasmid (Figure 1). By contrast, OVCAR5 presented overexpression of SYTL2 and therefore our target gene was deleted in these cells through the CRISPR editing system (data not shown). In addition, SKOV3 and CAOV3 cells were transfected with plasmids expressing different mutant forms of SYTL2 (Figure 1).

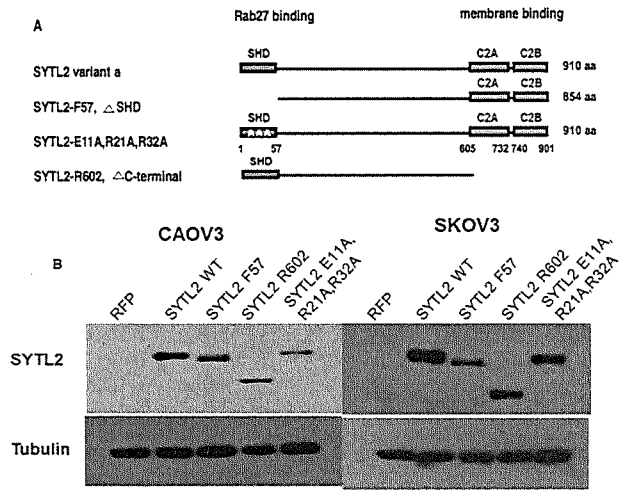

Figure 1: a) schematic representation of SYTL2 mutant genes that were originated in our lab to study the function of this gene; **b)** overexpression of SYTL2 and three different mutant forms of these gene in CAOV3 and SKOV3 cells (RFP= non- transfected control).

Major Task 3: Test how SYTL2 induces cells behavior changes in vitro. We have then tested how changes in SYTL2 expression changed in vitro behavior of cells, specifically regarding cells proliferation, migration and chemoresistance. Indeed, the preliminary data presented in the grant application showed that overexpression of SYTL2 in A2780 cells induced cells proliferation, migration, and chemoresistance, thus providing rationale to our genomics analysis indicating that amplification and overexpression of SYTL2 correlated with poor survival in patients with advanced-stage/high-grade ovarian cancer. However, we could not reproduce our preliminary data in the CAOV3 and SKOV3 cells overexpressing SYTL2; no difference in behavior was observed between the wild type cells and the clones expressing SYTL2. Surprisingly, also deletion of the endogenous SYTL2 gene in OVCAR5 cells did not induced any changes of cells in vitro behavior (Figure 2).

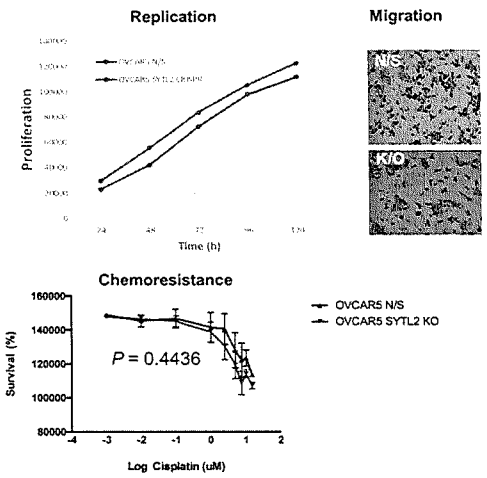

Figure 2: analysis of OVCAR5 cells in vitro behavior following deletion of SYTL2. No differences were observed in either cells proliferation (upper left graph), or cisplatin resistance (bottom left graph), or migration (microphotographs of cells migrating through a Boyden chamber) when comparing OVCAR5 non transfected (n/s) control clone with SYTL2 knock out (k/o) clone.

Major Task 3: Test how SYTL2 induces cells behavior changes in vivo. Changes of cancer cells behavior are often subtler, and therefore more difficult to detect, compared to changes of behavior in vivo. We have thus injected the mice peritoneum with either the wild type or edited OVCAR5 cells to test the effects of SYTL2 deletion on tumor growth. Surprisingly, deletion of the gene caused strong induction of tumor growth (Figure 3), thus suggesting that in these cells SYTL2 acts as a tumor suppressor. This was an unexpected result whose significance is currently being investigated.

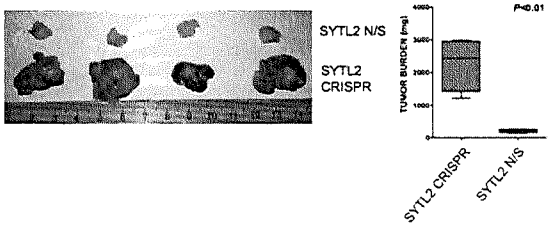

Figure 3: analysis of OVCAR5 cells in vitvo behavior following deletion of SYTL2. Mice injected with OVCAR5 depleted for SYTL2 (SYTL2 CRISPR) formed much larger intraperitoneal injections compared to those injected with cells carrying the wild type gene (non transfected n/s). The left images show representative images of tumors extracted from mice; the right graph shows the average and standard error of tumors sizes extracted from the different mice groups. N=8, tumors were grown for 3 weeks before analysis.

Additional data and conclusions: During the past year, the lab has generated a broad number of ovarian cancer patients derived xenografts (PDXs) which generate tumors in mice models that largely maintain the genomics features of the patients' cancers. We have thus used these PDXs to test endogenous expression of SYTL2. Surprisingly again, SYTL2 was not expressed in 9 of 10 PDXs, which further contradicted our preliminary genomics data.

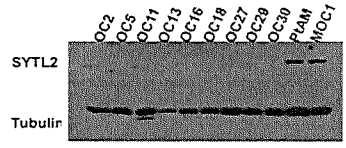

Figure 4: western blot analysis showing expression of SYTL2 in 10 PDX cells generated at MGH (OC2, 5, 11, 13, 16, 18, 27, 29, 30, PtAM), and in an ovarian cancer mouse cell line (MOC1). Tubulin was used as loading control.

Results disseminated to communities of interest: Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them: The current grant is being transferred from MGH to UAB. Throughout this transfer the studies will be on hold and this may delay the accomplishment of the project in year 2. In addition, given the contrasting data we have achieved more unexpected analysis will have to be performed, which may also delay the full accomplishment of the project.

IMPACT

Impact on the development of the principal discipline(s) of the project: Identifying and characterizing oncogenes and tumor suppressors of ovarian cancer is fundamental to improve management of this disease.

Impact on other disciplines: Nothing to report

Impact on technology transfer: Nothing to report

Impact on society beyond science and technology: Nothing to report