CAF-derived MFAP5 is a novel transcription regulator for immune checkpoint CD47 in ovarian cancer

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Background

High-grade serous ovarian cancer (HGSC) is the most widespread type of epithelial ovarian cancer, accounting for approximately 75% of all epithelial ovarian cancers[1]. As one of the most common types of cancer worldwide, HGSC also poses as a significant source of mortality amongst patients diagnosed in advanced stages. Despite a high response rate for platinum/taxane-based chemotherapy that is commonly used to treat HGSC, most patients develop chemoresistance[2]. The tumor microenvironment (TME), composed primarily by fibroblasts, endothelial cells, lymphocytic infiltrates and extracellular matrix proteins, can directly affect cancer cell growth, migration, and differentiation1, thereby presenting a unique aspect of diagnosing and treating cancer. Cancer associated fibroblasts (CAFs) are primarily responsible for producing the structural components of the stromal microenvironment, which is mostly composed of collagen type I, II and IV as well as fibronectin1[2]. CAFs also produce secreted factors such as cytokines and growth factors, which maintain normal tissue homeostasis by signaling to other cell components in the stroma, such as immune, fat, vascular, smooth muscle and epithelial cells. Moreover, CAFs have been recently shown to play a key role in modulating the malignant phenotypes of HGSC by producing factors such as growth factors and cytokines that influence the tumor microenvironment (TME).

Microfibrillar-associated protein 5 (MFAP5) has recently been shown by Mok et al. and others to be upregulated in CAFs of several tumor types including non-small cell lung cancer, pancreatic cancer, ovarian cancer, prostate cancer, and breast cancer[3]. In particular, over-expression of MFAP5 in CAFs associated with poor prognosis in ovarian cancer. MFAP5 has an Arg-Gly-Asp (RGD) binding motif that can bind αvβ3 integrin to enhance angiogenesis and metastatic potential of HGSC cells through the activation of calcium-dependent FAK/ERK/LPP and FAK/CREB/TNNC1 pathways, respectively[4]. Silencing MFAP5 in CAFs using MFAP5 specific siRNAs suppressed ovarian cancer growth and metastasis, and angiogenesis *in vitro* and *in vivo*. Our preliminary data showed that exogenous MFAP5 increased CD47 mRNA expression in ovarian cancer cells. We hypothesize that that CAF-derived MFAP5 can transcriptionally up-regulate CD47 in ovarian cancer cells. To identify the molecular mechanism by which MFAP5 regulates CD47 expression, we analyzed the promoter sequence of CD47.

Methods and Results

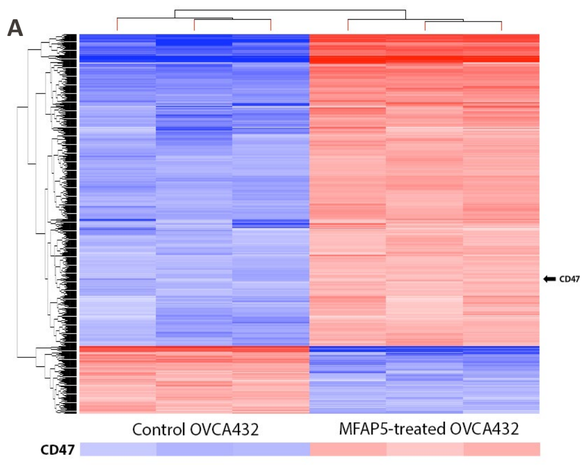

Figure 1. MFAP5 upregulates CD47, an immune checkpoint mediator in ovarian cancer cells. **A**. Transcriptome analysis on MFAP5-treated ovarian cancer cell OVCA432 to identify differentially expressed immune-related genes induced by MFAP5. **B**. Correlation between MFAP5 mRNA expression in microdissected CAFs and CD47 mRNA expression in microdissected ovarian cancer cells, indicating that CAF-derived MFAP5 may play a role in regulating CD47 expression in ovarian cancer cells (R=0.428, P=0.002, N=52). **C-D**. qRT-PCR analysis (**C**) and Flow Cytometry intracellular staining (**D**) of CD47 expression levels showed a significant increase in both mRNA and protein expression of CD47, after 48h treatment with rMFPA5, compared with PBS-treated control cells. *, P< 0.05; **, P< 0.01.

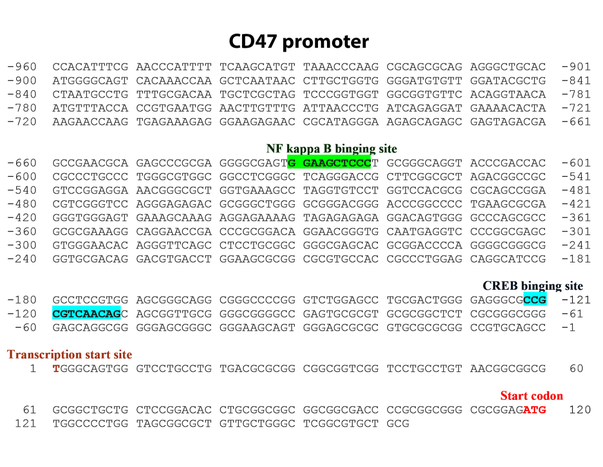

Figure 2. Diagram showing the promoter sequence of CD47 with NF-κB and CREB binding sites highlighted. To identify the molecular mechanism by which MFAP5 regulates CD47 expression, computational analysis of the promoter sequence of CD47 was performed using MatInspector, TESS, and TFSEARCH software. The results revealed that the CD47 promoter has a CREB and an NF-kB consensus binding sequence. Since promoter analyses of CD47 showed a potential CREB binding site and since our previous findings showing that binding of MFAP5 to αvβ3 integrin activates the calcium-dependent FAK/ERK/CREB/TNNC1 signaling network in ovarian cancer cells, we hypothesize that activation of the MFAP5/αvβ3 integrin/FAK/ERK/CREB signaling network also plays a role in up-regulation of CD47 in ovarian cancer cells[4].

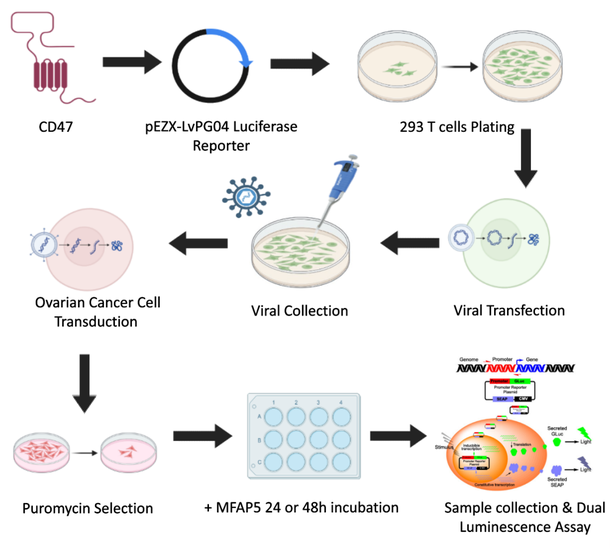

Figure 3. Cartoon representing workflow for viral production and luciferase (reporter) assay. CD47 promoter sequences with wild-type or mutated putative CREB binding sites were cloned into the pEZX-LvPG04 luciferase reporter vector. Plasmids were then transfected into 293 T cells plated in 10cm dishes for packaging purposes. The 293 T cells then produced a virus with the CD47 promoter sequences which was collected. The virus was used to transduce the ovarian cancer cells to express the CD47 WT or mutated promoter sequences. 48h after viral transduction, puromycin was added to the cancer cells to select only transduced cell clones. After 2 weeks of selection, cancer cells were utilized for downstream analyses. In a 12 well plate, the three cancer cell lines OVCA 420, OVCA 433, PEA.1 were treated with MFAP5 for 24 or 48 hours. The supernatant was then collected and used in a dual luminescence assay (GeneCopoeia).

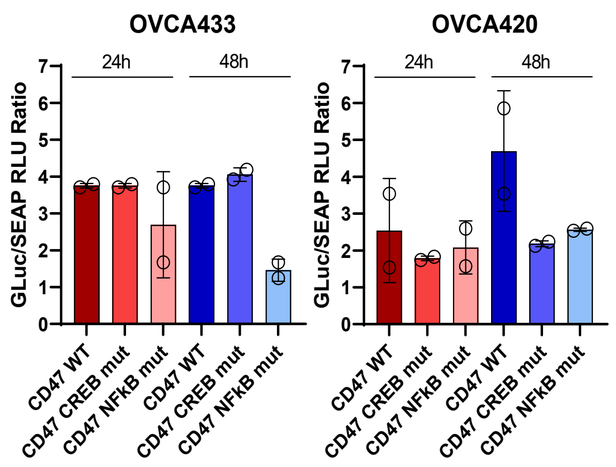

Figure 4. Luciferase Assay. Cell lines, OVCA 420 and OVCA 433, able to over express CD47 at both protein and mRNA levels after treatment with recMFAP5 (Figure1 C-D) were chosen for the luciferase assay. After stable transduction with CD47 promoter WT, CREB mutated or NF-kB mutated (Figure 3), Cell lines were treated for 24 or 48h with recMFAP5 at 200ng/ml and supernatant was then collected. Luciferase assay was performed according to manufacturer's indications. The GL-H buffer was used to ensure ample production of data due to the GL-H buffer's high sensitivity. Data are represented as the ratio between Gluc and SEAP signals, and samples were run in duplicates. Both cell lines transduced with CREB or NF-kB mutated plasmids show a decreased binding activity signal compared to cells transduced with the WT plasmid.

Conclusions

- Stromal MFAP5 mRNA expression correlates with CD47 mRNA expression in HGSOC tissue samples.
- MFAP5 up-regulates CD47 mRNA and protein in ovarian cancer cell lines.
- MFAP5 responsive elements on the CD47 promoter consist of a CREB and a NF-κB binding sites in ovarian cancer cells.
- Future study to delineate the singling network involved in transcriptionally up-regulation of CD47 by MFAP5 is warranted.

References

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