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Selection of aptamers against Jagged-1 protein

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1. Introduction

According to the World Health Organization, breast cancer has the highest incidence and mortality among women worldwide [1], [2]. The Jagged-1 (JAG1) protein plays an important role in the biology and development of cancer, influencing angiogenesis, growth of neoplastic cells, tumor stem cells, epithelial-mesenchymal transition, metastatic processes and resistance to therapies in various types of cancer. To date, few studies have addressed the production of therapeutic agents aimed at inhibiting JAG1. Thus, the development of tools that allow the *in vivo* diagnosis of the JAG1 ligand in patients with tumor, as well as alternatives for a therapy for this ligand are extremely important [3], [5]. Therefore, in this project, our aim was to select an aptamer for JAG1 ligand using an aptamer library, that could be used as a radiopharmaceutical for PET / SPECT / CT diagnosis of tumors that express JAG1. This aptamer has the potential to be radiolabeled with diagnostic radionuclides (eg. $^{99m}Tc/^{18}F$) or therapeutic radionuclides (eg. ^{177}Lu).

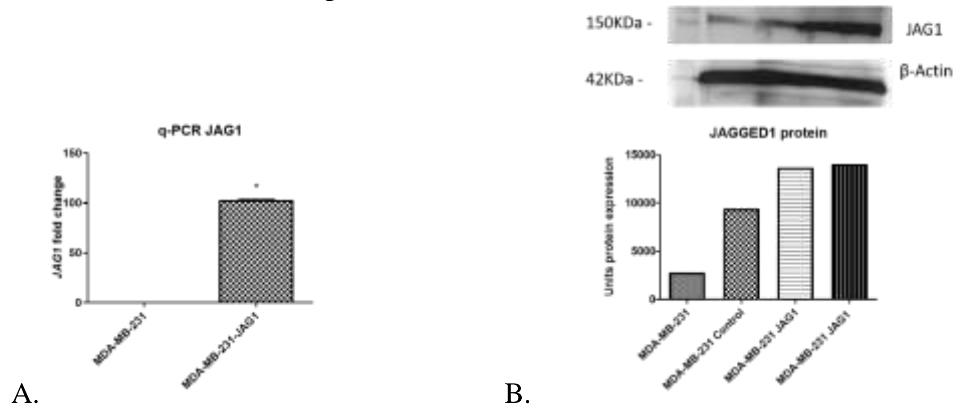
2. Methodology

The full length JAG1 protein was overexpressed through DNA lentiviral transfection in HEK293T cells followed by viral transduction in the breast cancer cells MDA-MB-231 (MDA-MB-23-JAG1). An empty vector was used as a control (MDA-MB-231-Control). The selection of transduced cells was made with blasticidin. To confirm the successful expression of JAG1, RT-PCR and Western Blot analysis were performed. $5x10^6$ MDA-MB-231 overexpressing cells or control cells were further inoculated in Balb/c nude mice and the tumor volume was measures with a caliper every two days to evaluate tumor growth. Tumor volume was quantified by the formula 0.5 x larger diameter x smaller diameter^2. The *in vivo* experiment was authorized by the IPEN ethics committee under number n° 218/18.
The selection of a specific aptamer for JAG1 was performed using the SELEX (Systematic Evolution of Ligands by Exponential Enrichment) method. For that, a library of ssDNA aptamers linked with FAM fluorochrome was used to select specific aptamers for JAG1 binders. The selction was made alternating the incubation cycles of the ssDNA aptamer library linked with FAM fluorochrome with MDA-MB-231 cells or MDA-MB-23-JAG1. The immunofluorescence assay was made with the incubation of fluorescently labelled (FAM) aptamers (2 µM) from cycle 1 or 3 of selection or the full library in MDA-MB-231 and MDA-JAG1 cells at 4 °C for 1 h. After incubation, the cells were washed with PBS and photographed under an immunofluorescence inverted microscope Nikon Ts 100.

3. Results and Discussion

In this study, JAG1 was efficiently overexpressed in MDA-MB-231 cells. We could observe a significantly increased mRNA and protein levels of JAG1 in MDA-MB-231-JAG1 cells when compared to MDA-MB-231

cells or MDA-MB-231-Control cells (Figure 1).



A. B.

Figure 1: The ligand JAG1 was overexpressed through DNA lentiviral transfection in the breast cancer cells MDA-MB-231. After Blasticin selection, (A) total RNA was extracted and *JAG-1* mRNA levels were evaluated by Real-Time PCR. Relative quantification was done using the ΔΔCt method normalizing to GAPDH gene expression. Additionally, (B) total protein was isolated and the protein levels of JAG-1 were assessed by Western blot. β-actin was used as a loading control. Data are the mean (S.D.), n = 3, *$p < 0.05$, by two-tailed unpaired Student's t-test.

MDA-MB-231-JAG1 or MDA-MB-231 cells were next subcutaneously injected in Balb/c nude mice. We could observe that the overexpression of JAG1 significantly increases the growth of MDA-MB-231-JAG1 cells when compared to MDA-MB-231-Control cells (Figure 2).

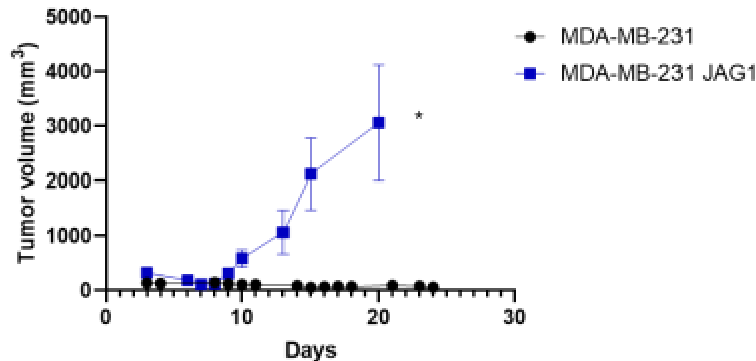


Figure 2: Tumor growth curve showing the tumor volume of MDA-MB-231-Control and MDA-MB-231-JAG1 cells. Data are the mean (S.D.), n = 5. Data were analyzed by non-linear regression. *$p < 0.05$, by two-tailed unpaired Student's t-test.

The SELEX assay was done using MDA-MB-231-Control cells as a negative control cycle and MDA-MB-231-JAG1 as a positive control cycle. All positive cycles had a good extraction efficiency with a yield of 248.9 ng/ul in the third cycle (Table 1). The negative cycles were discarded and positives cycles were stored for further purification and characterization.

Table 1: SELEX aptamer cycle.

Cycle – SELEX	N° of cells	tRNA (25ug/ul)	Washing with selection buffer 1x	Pool Concentration	Incubation Time	Quantification
1	10^6	0	0	100 uM	40min	288,5ng/ul
2	10^5	1ul	3x	10 uM	40min	46,1ng/ul
3	5×10^4	2ul	6x	10 uM	30min	248,9ng/ul

The immunofluorescence assay showed that the selected aptamers against MDA-MB-231JAG1 cells from the third cycle of SELEX (Table 1) were able to bind MDA-MB-231-JAG1 cells more specifically than the selected aptamer from the first cycle of SELEX or the aptamer library. Besides that, at 40-fold magnification, we also found that aptamer binds to cell membrane, cell cytoplasm and small vesicles within cells (Figure 3).

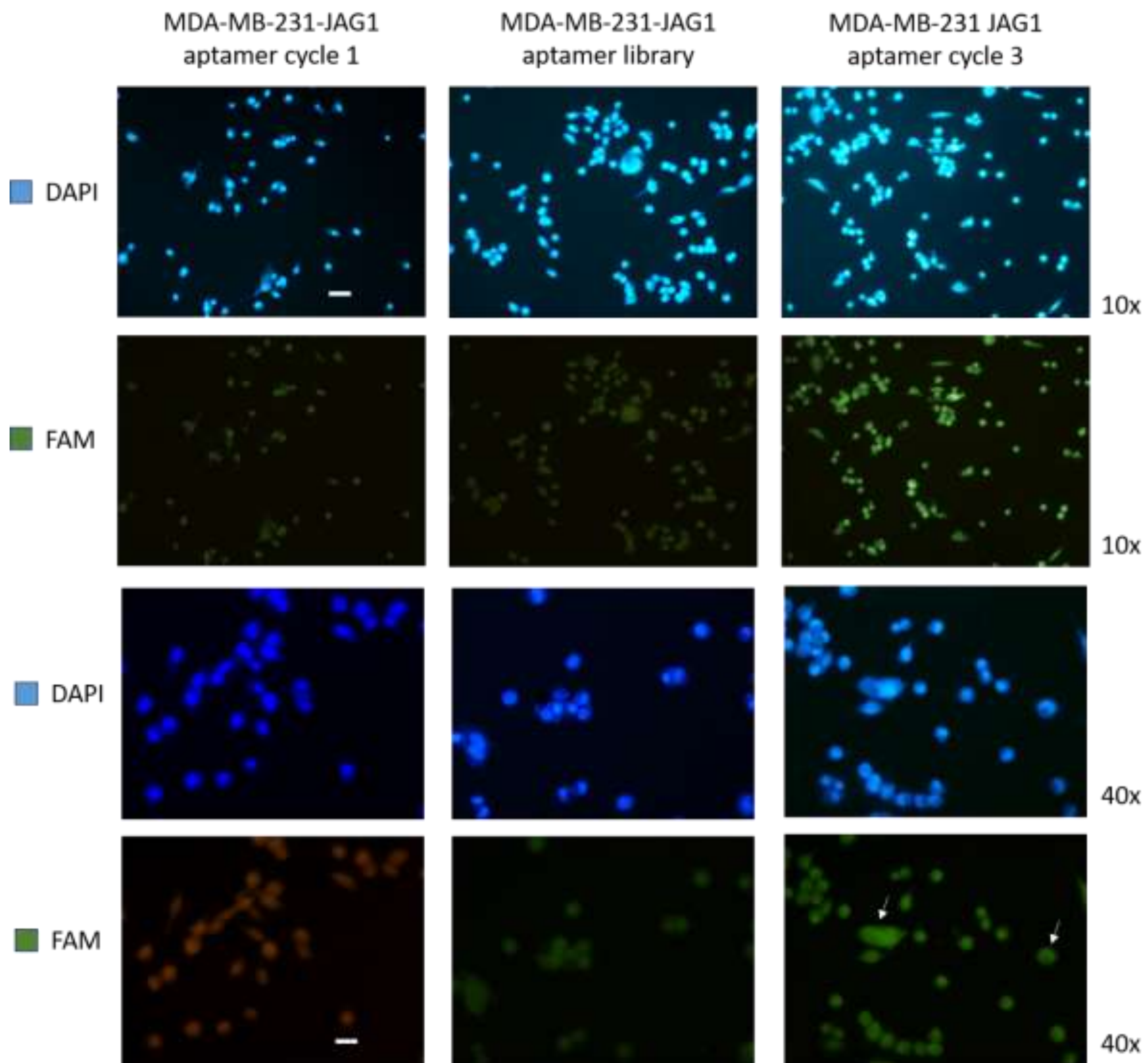


Figure 3: MDA-MB-231-JAG1 in a monolayer were fixed in 4% PFA and then FAM-aptamers from cycle 1, 3 or the library were incubated with cells. Representative immunofluorescence images (10X or 40X magnification) showing JAG1 aptamers (green) and nucleus (blue) staining are presented. White arrow shows cytoplasmic vesicles. Scale bar, 200 µm for 10x and 800 µm for 40x of magnification.

Altogether our results demonstrate the selection of a new molecule based on ssDNA aptamers able to specifically bind to breast cancer cells that overexpress JAG1 protein. This aptamer has the potential to be radiolabeled with diagnostic or therapeutic radionuclides (eg ^{99m}Tc, ^{18}F or ^{177}Lu). Indeed, several studies have demonstrated that aptamers have the potential to be used as therapeutic or diagnostic agents as AS1411 and MAG3-apt radiopharmaceuticals through their radiolabeling with radionuclides and their affinity with molecular targets [6]–[8].

4. Conclusions

Our work showed that MDA-MB-231-JAG1 cells overexpress more mRNA and JAG1 protein than control cells (MDA-MB-231-Control), being an important tool for the study of JAG1 biology in tumors. We also selected aptamers with high affinity for MDA-MB-231-JAG1 cells that could be a useful tool for the development of new radiopharmaceutical for the diagnosis and treatment of tumors that overexpress JAG1.

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