



GT75 APTAMER AGAINST EUKARYOTIC ELONGATION FACTOR 1A AS POTENTIAL ANTICANCER DRUG FOR CASTRATE-RESISTANT PROSTATE CANCER (CRPC)

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Abstract — Prostate cancer diagnosis is increasing, being the second most frequently cancer in men worldwide. The treatment of castrate-resistant prostate cancer is often unsuccessfully and new therapeutic interventions are searching for. Nucleic acid aptamers targeting eEF1A proteins are emerging molecular tools for the control of cancer growth. We found that an aptamer named GT75 was able to bind to eEF1A proteins of human prostate cancer cell lines and to significantly and specifically reduce their growth with respect to the control oligomer CT75. The highest anti-proliferation effect was found in the androgen-independent PC-3 cells. Interestingly, GT75 was able to specifically inhibit the migration of PC-3 cells but not that of the non-tumorigenic PZHPV-7 cells. The overall results suggest that the GT75 aptamer targeting eEF1A proteins is a promising molecular drug to develop for the control of the castrate-resistant prostate cancer.

Index Terms — Prostate cancer cell lines, castrate-resistant prostate cancer, aptamers, eEF1A, anti-proliferation effect

1 BACKGROUND

Prostate cancer is a significant medical burden in men in the Western world being the second most frequently diagnosed cancer and the sixth leading cause of cancer death in males: 14% of the total new cancer cases and 6% of the total cancer deaths worldwide. It has been estimated that the year 2013 witness 238,590 new prostate cancer cases and 29,720 prostate cancer deaths (1). Although surgery, radiation, or both, can be curative for patients with localized disease, a significant proportion of these patients have recurrent disease which can lead to the development of metastases, especially in the high risk group – a transition to the lethal stage of the disease. Androgen depletion is the standard therapeutic treatment but after a period of stability, the tumor regrowth as castration-resistant disease (15-20% of cases) (1). Castrate-resistant prostate cancer (CRPC) has limited therapeutic interventions and the survival is poor. The median survival of patients with metastatic CRPC is 12–16 months from the time of diagnosis to death (2). No curative treatments are available at this stage of the disease.

Aptamers are nucleic acids selected to bind any molecules, protein included, in order to affect the biological activity of the cognate target that are raising as diagnostic and therapeutic tools (3). GT aptamers recognizing eukaryotic elongation factor 1A are single-stranded DNA strands discovered in 2002 as anti-proliferative agents in hematopoietic human cancer cell lines, not affecting the growth of normal cells, that are able to increase the therapeutic index of conventional anti-cancer drugs too (4, 5).

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2. Heidenreich A, Schrader AJ. The treatment of hormone refractory prostate cancer. *EAU Update Series.* 2003;1:40–50.
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4. Dapas B, Perissin L, Pucillo C, Quadrifoglio F, Scaggiante B (2002) Increase in therapeutic index of doxorubicin and vinblastine by aptameric oligonucleotide in human T lymphoblastic drug-sensitive and multidrug-resistant cells. *Antisense Nucleic Acid Drug Dev* 12 (4): 247-255.
5. Dapas B, Tell G, Scaloni A, Pines A, Ferrara L, Quadrifoglio F, Scaggiante B (2003) Identification of different isoforms of eEF1A in the nuclear fraction of human T-lymphoblastic cancer cell line specifically binding to aptameric cytotoxic GT oligomers. *Eur J Biochem* 270 (15): 3251-3262.

2 APPROACH & METHODS

General approach

To study the effect on cell growth/motility of a GT75 aptamer in in vitro human prostate cancer model, cell lines with different differentiation and androgen responsiveness phenotypes (LNCaP, 22Rv1, PC-3) were used and compared with a non tumorigenic control cell line (PZHPV-7)

Methods

Immunofluorescence; Proliferation assay test (MTT); Scratch test; Western-blotting, UV-crosslinking assay; FACS

What new product or service might be realised thanks to your results?

New therapeutic intervention for the treatment of CRPC

Have you realised a prototype? No

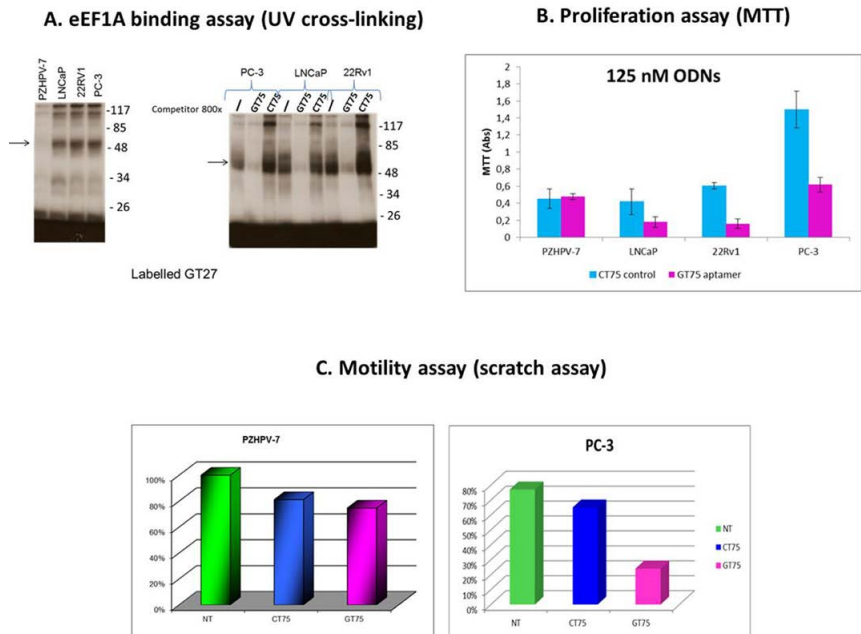


Figure 1. Effects of the GT75 aptamer compared to the control oligomer CT75. A) Binding of GT75 to eEF1A proteins that is marked by the presence of the 50 kDa band (arrow) in cellular extracts of tumorigenic (LNCaP, 22RV1 and PC-3) and non-tumorigenic (PZHPV-7) human prostate cell lines; B) Effect of GT75 on the cellular growth of the human prostate cancer cell lines and of the non-tumorigenic cells (PZHPV-7); C) Effect of the GT75 on the migration of the PC-3 cells with respect to the non-tumorigenic PZHPV-7 cells.

Contacts/collaborations needed

To do what: cell biology and molecular biology collaborations for highlighting the basic mechanism of action of GT75 aptamer in prostate cell lines, especially regarding the interactions of GT75 with eEF1A isoforms and the modulation of genes and miRNA expression by GT75 treatment. Collaborations on the in vivo effect of GT75 in CRPC animal models will be welcome.

Communication tools/strategy

Your 3 best scientific publications:

Scaggiante B, Dapas B, Farra R, Grassi M, Pozzato G, Giansante C, Fiotti N, Tamai E, Tonon F, Grassi G. Aptamers as targeting delivery devices or anti-cancer drugs for fighting tumors. *Curr Drug Metab.* 2013 Jun;14(5):565-82.

Scaggiante B, Dapas B, Bonin S, Grassi M, Zennaro C, Farra R, Cristiano L, Siracusano S, Zanconati F, Giansante C, Grassi G. Dissecting the expression of EEF1A1/2 genes in human prostate cancer cells: the potential of EEF1A2 as a hallmark for prostate transformation and progression. *Br J Cancer.* 2012 Jan 3;106(1):166-73.

Scaggiante B, Bonin S, Cristiano L, Siracusano S, Stanta G, Dapas B, Giansante C, Fiotti N, Grassi G. Prostate-tumor-inducing gene-1 analysis in human prostate cancer cells and tissue in relation to Mycoplasma infection. *Cancer Invest.* 2008 Oct;26(8):800-8.

Your patents: PD2010A000272-University of Trieste

Your websites (Your project, Researchgate, LinkedIn, etc): Researchgate and LinkedIn
Your Facebook: Yes

4 OBJECTIVE(S)

- Highlighting the mechanism of action of GT75 in human prostate cancer cell lines;
- Demonstrating the in vivo potential of GT75 in reducing tumor growth and in reversing drug-resistance in CRPC animal models
- Developing nucleic acid delivery methods based on polymeric and nano technologies for the in vivo release of GT75 for the in site treatment of CRPC

5 RESULTS

- By UV cross-linking competition assay we proved that a known eEF1A aptamer binder named GT27 was efficiently and specifically displaced by GT75 using LNCaP, 22Rv1 and PC-3 extracts. This validated GT75 use as eEF1A aptamer in prostate cancer cells (fig.A)
- By MTT assay we observed that GT75 was able to significantly reduce the growth of cancer cells in a dose-dependent manner at nmolar doses with respect to a control sequence named CT75. The anti-proliferative effect was minimal in androgen-dependent LNCaP cell line and maximal in the androgen-independent PC-3 cell line, resulting in: PC-3 > 22Rv1 > LNCaP. Notably GT75 did not significantly affect the growth of the non-tumorigenic PZHPV-7 cells with respect to CT75 control (Fig.B)
- By scratch assay we demonstrated that GT75 efficiently reduced the PC-3 cell migration but not that of the non tumorigenic control PZHPV-7 (Fig. C)
- Finally, we observed that GT75 was able to specifically increase the effect of conventional drugs such as bortezomib and idarubicin (not shown).

6 COLLABORATIONS

With other researchers (TRL0-4): Yes, those reported as co-authors

With SMEs (TRL5 -9)

With hospitals (TRL9): AOUTS- Cattinara-University of Trieste

With associations (TRL9): LILT (Italian League against Cancer)

Funds needed (forecast)

For basic research (investigation of biological mechanisms): € 20,000 consumables

For applied research (solutions for real-world problems): € 30,000 consumables + 30,000 (1 year fellowship)

For pilot & demonstrator activities (to develop a prototype) : € 20,000 consumables