



Development of a novel EGFR-targeted peptide for targeted delivery of drugs in anaplastic thyroid cancer

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I. Introduction

Anaplastic thyroid carcinoma (ATC) :

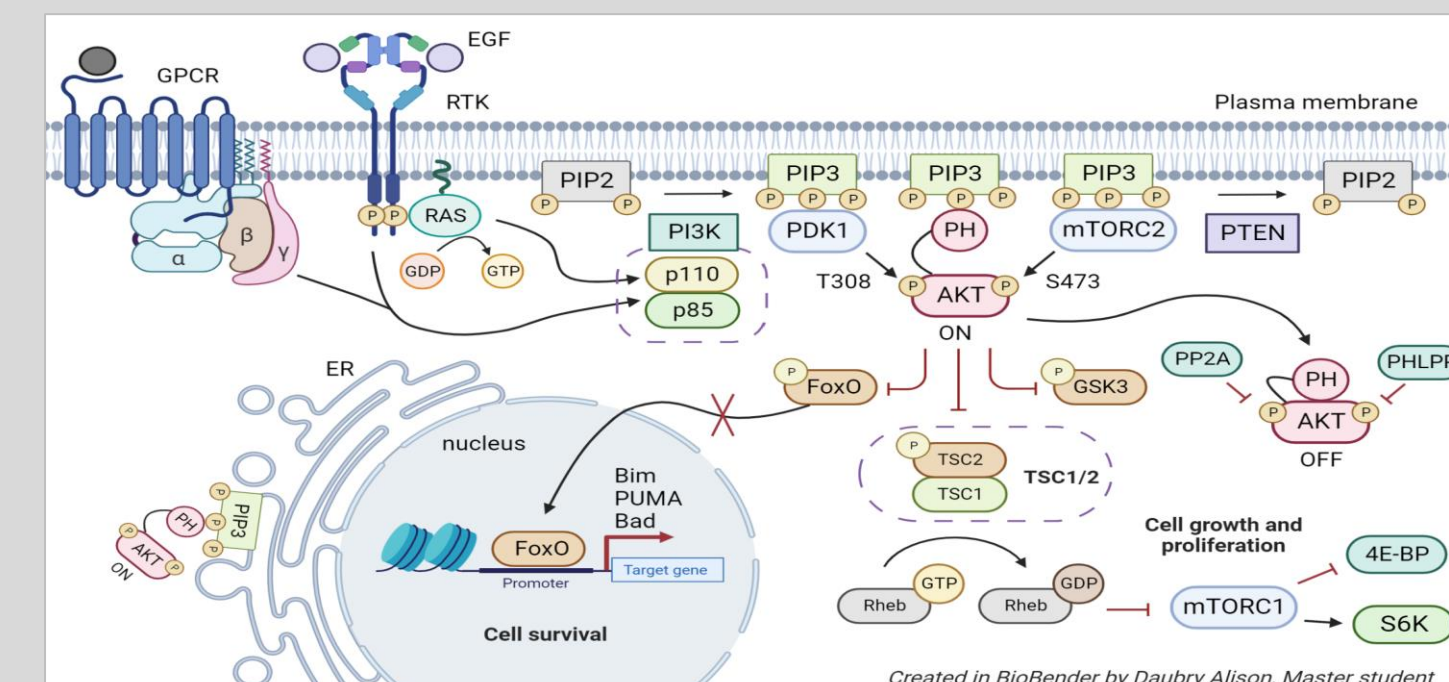
- aggressive and invasive type of thyroid cancer (TC)
- dismal prognosis
- median survival rate : 2 - 6 months after diagnosis [1]

Standard treatment : combination of surgery + ionizing radiation + chemotherapy
BUT chemotherapeutic agents → administered systemically
 many undesirable secondary effects
 ATC resistant to standard therapies [2].

a fundamental change of the therapeutic strategy's conception is required to manage this life-threatening oncologic disease.

- EGFR and PI3K/Akt/mTOR pathway represent **potential targets** for improved delivery and pharmacological action of chemotherapeutic agents [2,3].
 EGFR is indeed overexpressed in ATC
 and the PI3K/AKT/mTOR pathway is dysregulated.

Engineered peptides can be very helpful in this regard due to their lower toxicity and immunogenicity [4].



III. Material & method

Cell lines

The experiments were carried out on 8505c and Cal-62 ATC cell lines.

Nthy-ori 3-1 healthy cell line was used as control. For endocytosis studies by fluorescent microscopy, VP was coupled to rhodamine (VP-Rhod).

- **IF:** To evaluate the effect of VP on EGFR endocytosis and expression by immunofluorescence (IF) and Western Blot (WB), cells were grouped in four experimental conditions:

- (1) negative control in culture medium free of FBS,
- (2) positive control (1 μ M EGF),
- (3) test condition with 40 μ M VP or VP-Rhod,
- (4) test condition with 1 μ M EGF and 40 μ M VP or VP-Rhod

To corroborate the specific mechanism of endocytosis, VP was preincubated for 30 min with EGFR in solution before adding them to the conditions 3 and 4.

- **WB:** Total EGFR (EGFR_{tot}) expression was determined with EP38Y antibody, while phosphorylated EGFR (EGFRpY1068) was observed with EP774Y antibody (both from Abcam). For WB, the proteins blotted onto nitrocellulose membranes were imaged using a Bioimager Fusion FX (Vilbert, France) and were semi-quantitatively analyzed by densitometry.

The PC effects were investigated by IF (activated caspase 3) observation of apoptotic cells after incubation (30 min, 1h, 2h) with various concentrations (5, 10, 20 μ M) of PC.

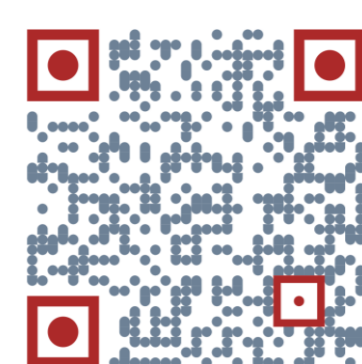
II. Objectives

Our ATC-targeted therapy is based on two peptides with different functions:

- A. Therapeutic peptide (TP)** induces apoptosis by blocking PI3K/Akt/mTOR signaling pathway;
- B. Vector peptide (VP)** targets EGFR and have the potential to be endocytosed and lead to the TP delivery to cancer cell. The peptide complex (PC) was synthesized by coupling PT and PV via a scaffolding small molecule. EGFR is naturally endocytosed after ligand binding and its intracellular trafficking was lately investigated in the context of receptor-mediated drug delivery.

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Any questions?
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IV. Results

EGFR is overexpressed and overactivated in ATC (**Fig. 1**).

Our VP is endocytosed into the cells independently of the presence of EGF and without activating the EGFR and thus the downstream signaling pathways.

Once in the cell, VP is more than **80% colocalized** with EGFR (**Fig. 3**)

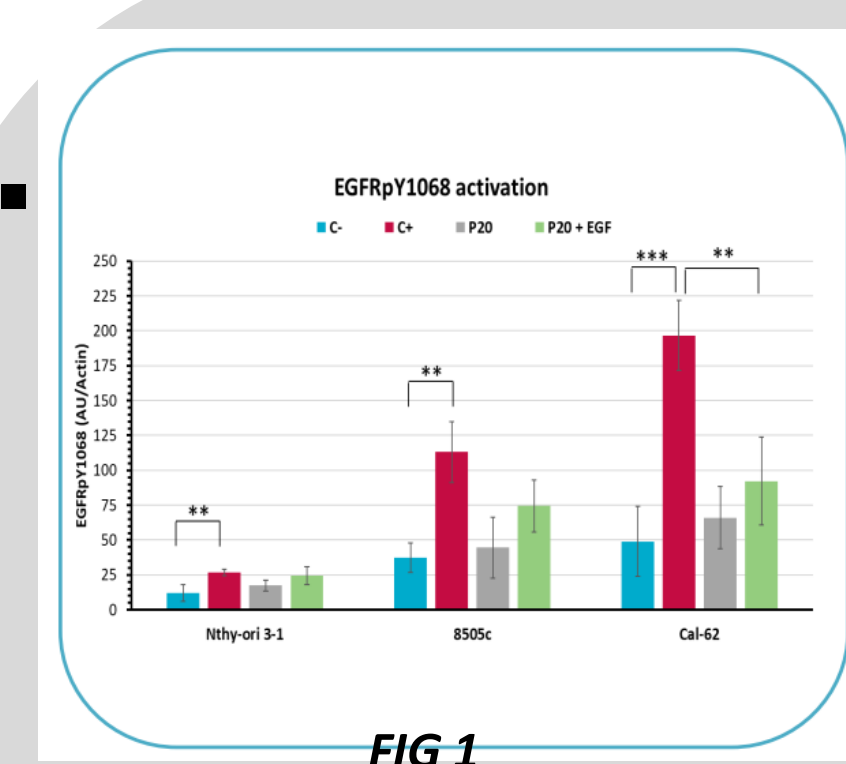


FIG 1

- The **preincubation with the EGFR in solution** has shown that the VP does not undergo competition in cells not stimulated with EGF and is still as much endocytosed in cells with competition as without competition (**Fig. 2**).
- On the other hand, there is a phenomenon of competition in cells **stimulated with EGF**, because **the VP is less endocytosed in cells with EGFR competition** than without competition (**Fig. 2**).

- **VP inhibits EGFR phosphorylation induced by EGF** at a level that is in the range of negative control and of P20 alone (**Fig. 1**). P20 could induce this effect when combined with EGF either by enhancing EGFR endocytosis, or by an antagonist effect produced on the receptor itself.

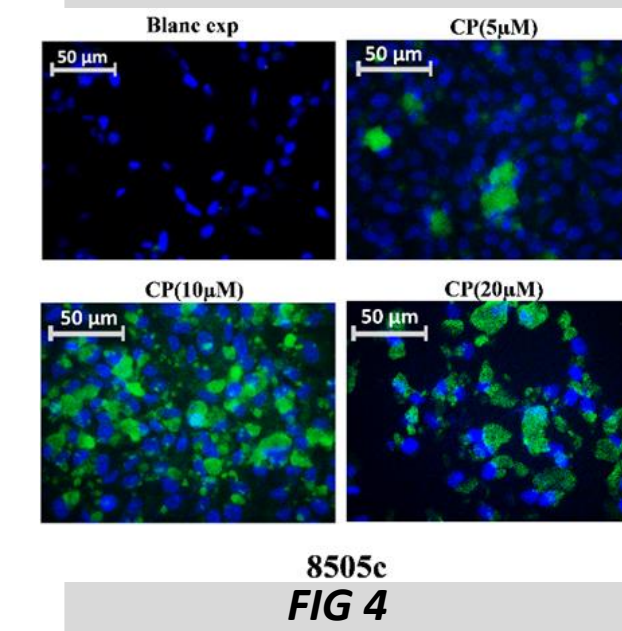


FIG 4

The **activation of caspase 3** has been observed by IF (**Fig. 4**) on the three **cell lines treated with PC**. Our previous studies revealed that BAD was activated by dephosphorylation following the inactivation of AKT by PC.
 We thus hypothesized that apoptosis might be triggered by the mitochondrial pathway

Based on these studies, an optimal PC concentration of **10 μ M (Fig. 5A and 5B)** and **1 hour** of incubation were identified as inducing the maximal apoptotic level (i.e., 100%).

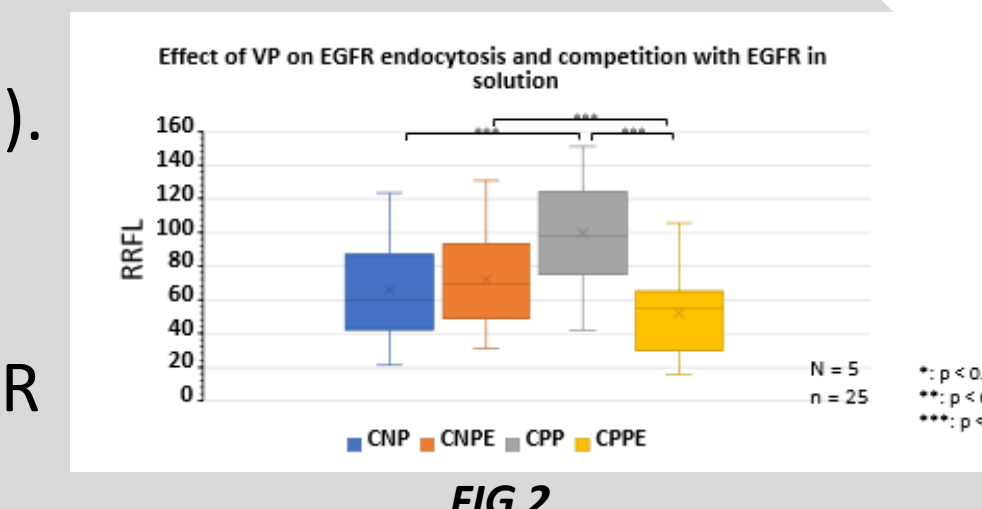


FIG 2

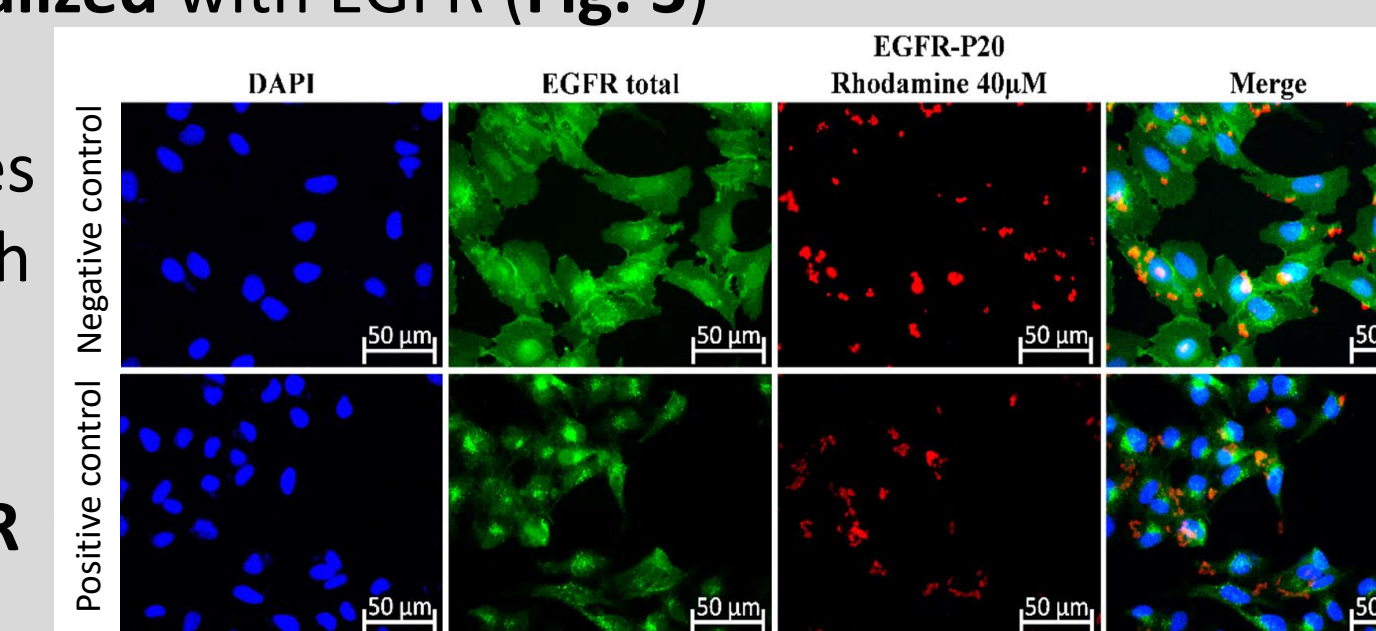


FIG 3

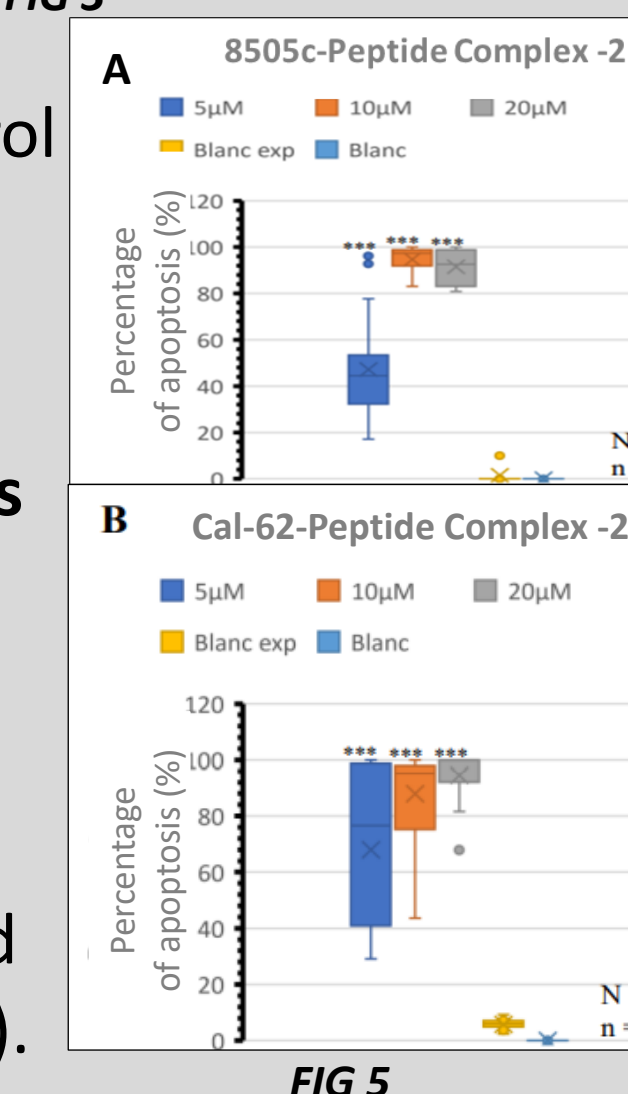


FIG 5

V. Conclusion

All these results confirm that the VP is a good candidate for targeting overexpressed and overactivated EGFR in ATC.

We can then conclude that the VP: (1) targets EGFR overexpressed by cancer cells to deliver the therapeutic peptide intracellularly; (2) induces EGFR endocytosis without activating it and without interfering with EGF binding; (3) VP is a non-competitive antagonist-type inhibitor of EGFR; (4) VP contributes to the therapeutic effect by decreasing the expression and activation of EGFR through its lysosomal degradation and, therefore, decreases the activation of the PI3K/AKT/mTOR pathway. All these effects make it possible to bring the TP intracellular to cancer cells while sparing healthy cells as much as possible.

References

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