Towards New GPER-selective Anticancer Probes

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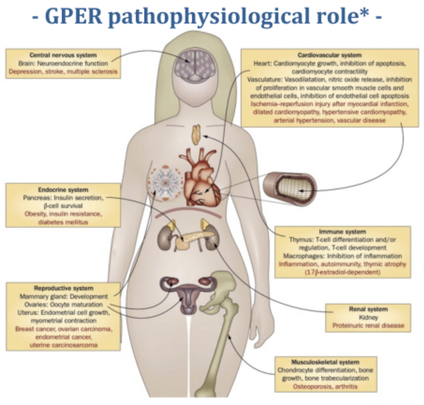
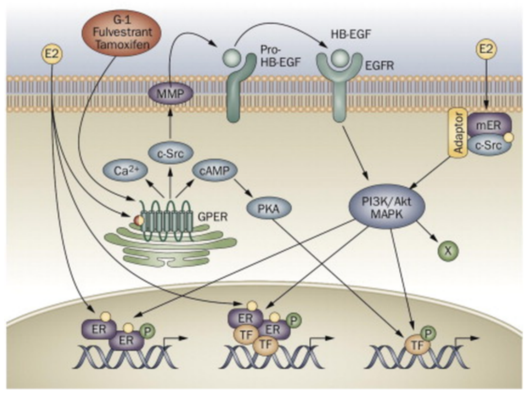
Background

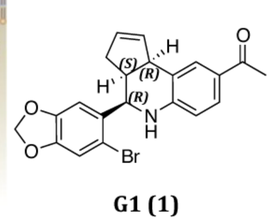
Together with ERα/β, GPER (G protein-coupled estrogen receptor 1, or GPR30) mediates important pathophysiological signaling pathways induced by estrogens and is currently regarded as a promising target for ER-negative and triple-negative breast cancer [1]. To better distinguish the role of GPER from nuclear ERα/β, selective ligands are essential. In this context **G1** (**1**) was proposed as a promising tool, being able to selectively bind GPER over ERα/β [2]. However, subsequent studies showed contradictory results on the effect of **G1** in the proliferation of cancer cells, leaving unsettled the need for better chemical tools [3-4].

- GPER pathophysiological role* -



- Signallings initiated by GPER and ER* -





G1 (1)

**Adapted from Molecular and Cellular Endocrinology 389 (2014) 71–83*

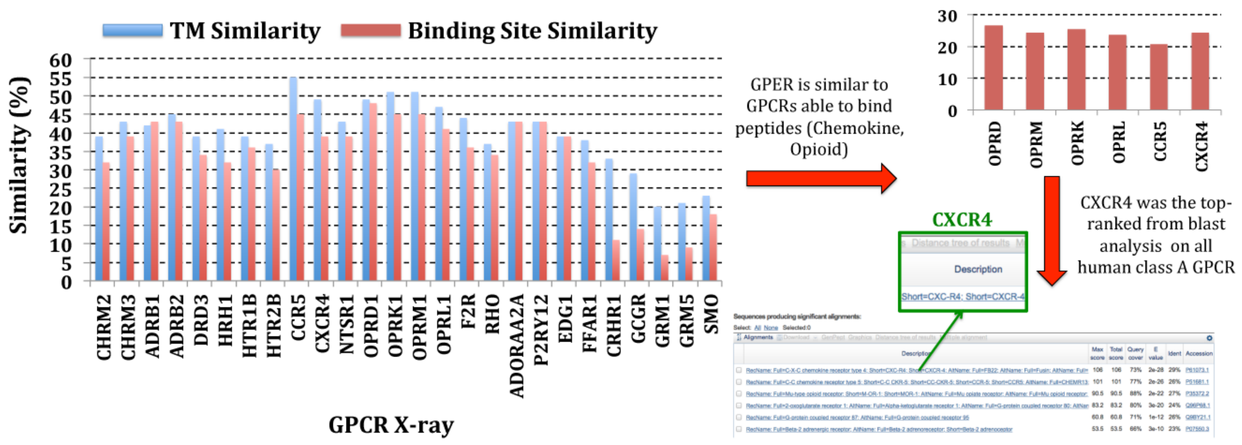
Aim

With the aim to **identify new GPER-selective anticancer probes**, we generated the GPER-**G1** complex that was refined by extensive MD simulations, and used to perform Virtual Screening studies. The top-ranked compounds were biologically evaluated in selected cell lines showing different level of GPER expression, such as MCF-7, SKBR-3 and HEK293.

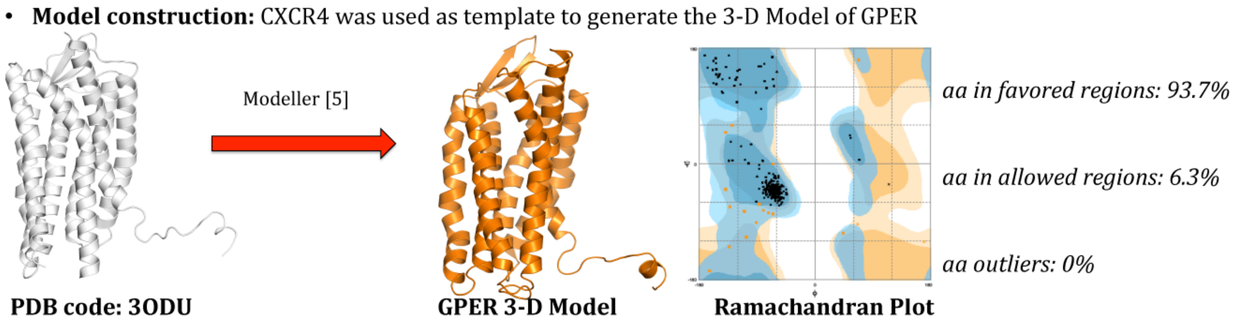
GPER 3-D Model

Several GPCRs X-ray crystal structures were solved so far, for class A, B, C and F. Unfortunately, for GPER no 3D structural information are currently available. Therefore, we decided to build a reliable model of GPER by means of homology modeling techniques.

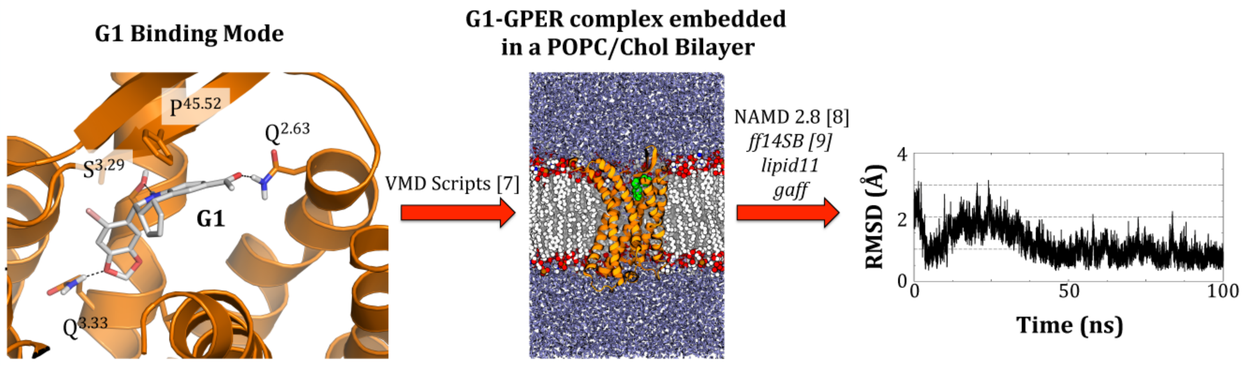
- **Template selection:** among the GPCRs X-ray crystal structures available we need to identify the closest GPER homologs, as templates.



- **Model construction:** CXCR4 was used as template to generate the 3-D Model of GPER



- **Model Validation: G1** was docked into GPER structure, by means of Glide [6], and the selected binding mode was evaluated by molecular dynamics simulations by means of NAMD 2.8.

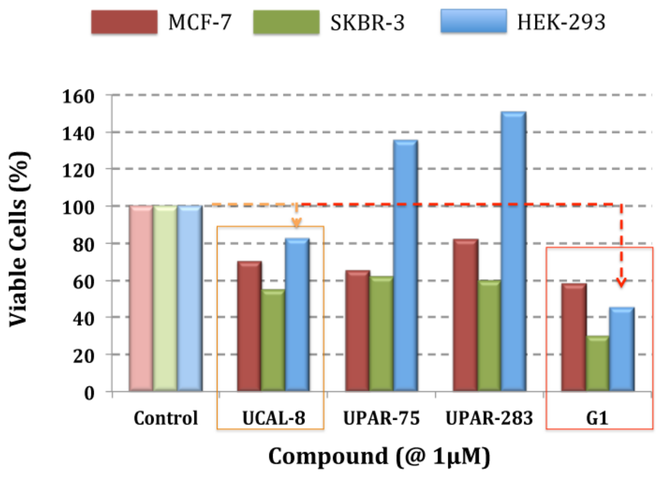


VS Protocol

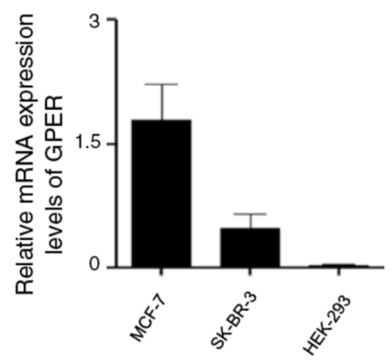
The refined **G1**-GPER complex was used to perform a Virtual Screening campaigns using in-house libraries (≈1000 compounds). The VS was performed by using the virtual screening workflow implemented in Glide [6]. The 10% top ranked compounds were initially selected, and among such compounds, only those showing a similar pattern of interaction with respect to **G1** were finally selected for biological evaluation (3 compounds).

Biological Assays

The selected compounds (UCAL-8, UPAR-75, UPAR-283) were evaluated for their **antitumor activity** in three different cell lines showing different level of GPER expression, such as MCF-7, SKBR-3 and HEK293.

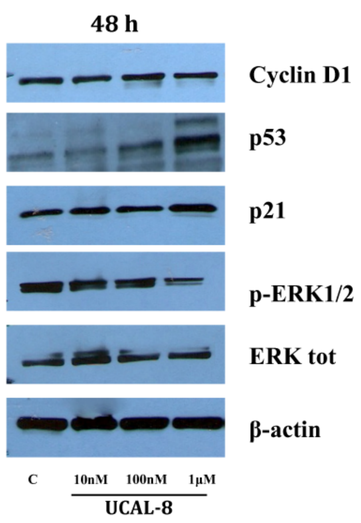


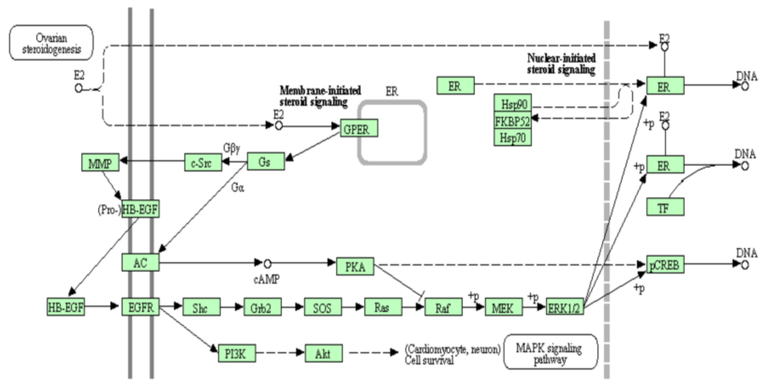
- mRNA GPER expression level in tested cell lines* -



**from J Cancer Res Clin Oncol (2014) 140:663–671*

- **MoA elucidation**: UCAL-8 was analyzed for its ability to activate specific intracellular effectors

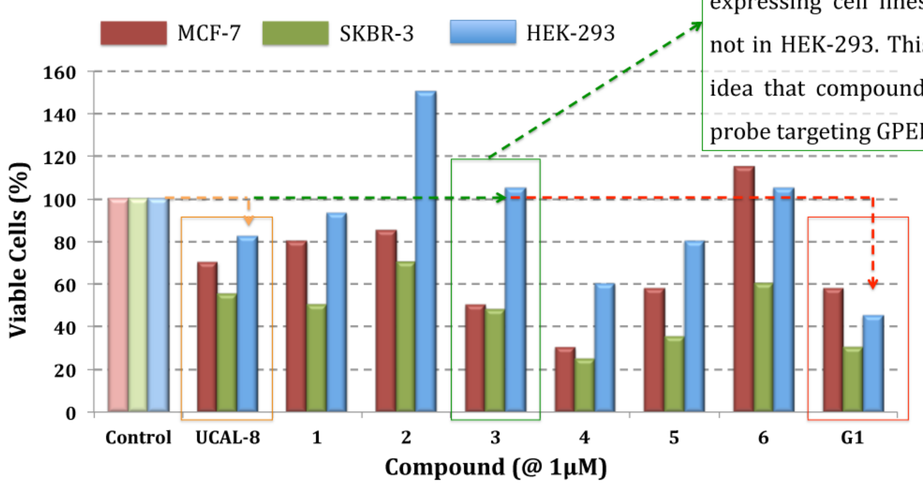




UCAL-8:

- Induces the reduction of p-ERK1/2
- Activates p53 (Apoptosis)
- Does not affect ERK tot., p21
- Does not act trough Cyclin D1 or β-actin

- **Hit-to-lead optimization**: structural modification of the hit compound (UCAL-8)



Compound **3** is inducing cell death in GPER-expressing cell lines (MCF-7 and SKBR-3), but not in HEK-293. This observations supports the idea that compound **3** is a selective antitumor probe targeting GPER.

Compounds **4** and **5** induced cell death also in HEK-293 cell lines, similarly to **G1**, while **6** resulted inactive in MCF-7

Conclusions

- The 3-D structural **model of GPER** was used to identify new chemical entities as modulator of the GPER receptor
- The 3-D model of GPER was validated by biological assays, leading to the **discovery of at least three new GPER modulators**
- Among the new GPER modulators identified, one (**UCAL-8**) is structurally related to **G1**, while **UPAR-75** and **UPAR-283** are structurally unrelated to **G1**, leading to the discovery of **two new chemotypes** as potential modulators of GPER
- In our assays **G1 induced cell death in a GPER-independent manner** (HEK-293), while **UCAL-8** effects on cell survival is tightly linked to the GPER expression even if its selectivity profile is not fully characterized yet .
- **UCAL-8 reduce the phosphorylation of ERK1/2** with no interference on cyclin D1 or β-actin, further supporting a GPER-dependent mechanism,
- Structural optimization of **UCAL-8** led to the **discovery of the lead compound 3**, which showed an improved activity profile and should be submitted to further biological investigation.

Future Perspectives

- The protocol herein described led to the discovery of two new chemotypes (**UPAR-75** and **UPAR-283**) as potential modulators of GPER, which will be further expanded to get clear SARs and to elucidate their MoA.
- The **lead 3** will be submitted to further biological investigation to clarify its MoA and define its biological potential.

References

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