Detection of multiple nuclear receptor–coregulator interactions in a single sample using MARCoNI PamChip technology

René Houtman, Dirk Pijnenburg, Diana Melchers & Rob Ruijtenbeek
PamGene International B. V., 's Hertogenbosch, The Netherlands

Study Design

Here, we present our next generation of high-content MARCoNI chips with a set of 155 immobilised peptides on each PamChip® array, which represent coregulator-derived motifs. Per array, the nuclear receptor/ligand conditions can be varied and *in vitro* interaction data for all 155 motifs are obtained within one hour. The principle and an example of nuclear receptor binding onto coregulator peptides immobilized on the PamChip® arrays are shown in figure 1. In figure 2 we show examples of the modulation of 15 different nuclear receptor ligand binding domains (Invitrogen) with their natural or synthetic ligands.

Key Findings

NR-coregulator interaction profiles were performed on 15 different NRs on 155 known NR coregulator proteins harboring either LXXLL (in coactivators) or LXXXIXXXL (in corepressors) motifs. Each of the NRs shows a different and specific modulation by its natural/synthetic ligand (figure 2).

"Author Quote"

An obvious application of this NR-coregulator interaction profiling platform is the screening at high definition of novel NR and their NR (ant)agonists.

Background

Cofactor recruitment is a crucial regulatory step in nuclear receptor (NR) signal transduction. The pathways towards gene expression involve ligand-dependent and independent interactions between NR and coregulator (CoR) proteins. In view of drug development, profiling these NR-CoR interactions is of importance to understand mechanisms of drug action, to steer drug specificity, to understand putative adverse drug effects (on- versus off target), and to tailor the pharmacotherapies.

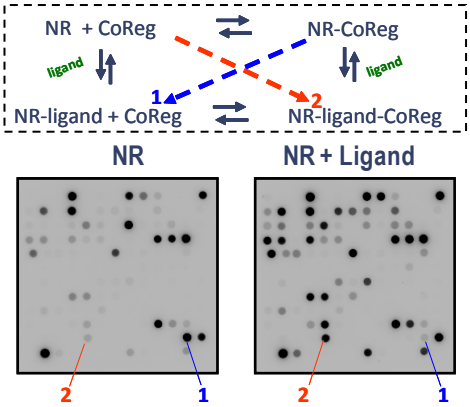

Figure 1: Schematic overview of Nuclear Receptor (NR) and Coregulator proteins binding as function of the ligand. The blue arrow (1) shows the effect of the ligand as an antagonist on a coregulator protein whereas the red arrow (2) shows the effect of the ligand as an agonist on a coregulator protein.

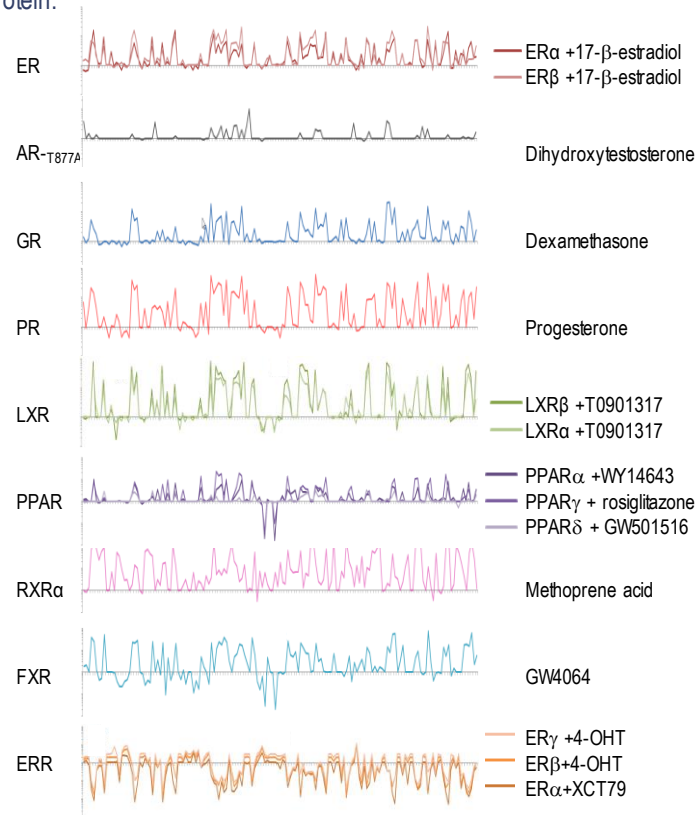

Figure 2: The modulation of 15 different nuclear receptors are shows as function of their synthetic of natural ligands across 155 coregulator proteins on the PamChip array. The modulation is the difference in binding of the ligand binding domain (LBD) of the nuclear receptor and the LBD-ligand complex.

References:

Houtman R., 2nd Benelux Nuclear Receptor Meeting, Netherlands 11-2009

Conclusion

MARCoNI technology, using dynamic PamChip® peptide microarrays, provides a powerful method for profiling of endogenous and ligand-dependent nuclear receptor-coregulator interactions.

Application note: #201015 (10-2010)

Contact us:
Wolvenhoek 10, 5211 HH Den Bosch, the Netherlands
☎+31 73 615 80 80, ✉ info@pamgene.com