Evaluation of various cell-based assays for olfactory receptors

Christina Kuhn1,2, Matthew Gibbs1, Scott McGreene3, Marcel Winnig1,2
1 Axxam SpA, Bresso (MI), Italy. 2 IMAX Discovery GmbH, Dortmund, Germany. 3 Bios Pasteur, WAK/THAM Centre for Per Nutrition, Melton Mowbray, UK.

Introduction

Our sense of smell plays an important role in our daily lives, especially in food consumption, influencing both food choice and the amount of food intake. The last three decades have seen great advances in the field of olfaction, beginning with the identification of olfactory receptors (ORs) and their signaling pathways. However, the pairing of individual ORs with their cognate ligands has been progressing slowly. This task has been complicated by difficulties in establishing a general functional assay system for ORs due to low expression levels of receptors in heterologous systems, high background signals of some ORs, and low reproducibility for others. We therefore set out to generate, evaluate, and compare different cell-based assays for ORs.

Results

Generation of reporter cell lines: activation of endogenous expression of RTP1s

Examples of OR responses in different cellular assays

Evaluation of various cell-based assays for olfactory receptors

Comparison of general performance of cell-based assays for ORs

Comparison of assay performance for individual ORs

Summary

- Using the CRISPR-Cas9 technology, we have generated two new reporter cell lines expressing the short 5’-arm of RTP1s (RTP1s) from its endogenous locus: PEAKrapid/RTP1s-endo and HEK293/NatClytin/CNG/RTP1s-endo. Both cell lines are suitable for functional expression of ORs.
- Using these two reporter cell lines, we have set up four cell-based assays for functional analysis of ORs: a CRE-NanoLuc reporter assay, a GloSensor assay, a PKA-NanoBiT assay, and our proprietary chAMPion assay.
- General assay performance was good for three of the four assays evaluated with only the PKA-NanoBiT assay providing a too low and unstable assay window.
- The other three assays, CRE-NanoLuc, GloSensor, and chAMPion, showed differences regarding noise, background, and sensitivity, also depending on the particular OR analyzed.
- While neither assay worked as the best assay for all ORs in general, a combination of two assays, CRE-luciferase and chAMPion assays, promises to yield good results for most ORs.

Examples of OR responses

Selected ORs and their ligands

<table>
<thead>
<tr>
<th>OR</th>
<th>Ligand(s)</th>
<th>Reference(s)</th>
</tr>
</thead>
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<tr>
<td>hOR5A1</td>
<td>2-bromo-3-phenylacetate</td>
<td>Mainland et al., Sci Data 2015; Jaeger et al., Curr Biol 2013</td>
</tr>
<tr>
<td>hOR5B1</td>
<td>Phenylacetate</td>
<td>Mainland et al., Sci Data 2015; Audouze et al., PLoS One 2014; Mainland et al., Sci Data 2015</td>
</tr>
</tbody>
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Examples of ORs used in the assay system for ORs due to low expression levels of receptors.

Examples of OR responses

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