Characterization of olfactory receptors expression in BON cells

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CHARACTERIZATION OF OLFACTORY RECEPTORS EXPRESSION IN BON CELLS

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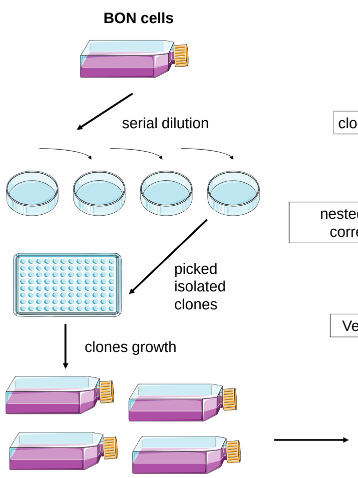
BON cells are a cell line derived from human enterochromaffin cells. They were previously described to express various olfactory receptors (ORs), but it is unclear whether a single cell expresses several types of ORs, as reported for spermatogenic cells, or a single type of OR as for olfactory sensory neurons (OSNs). The goal of the present work was first to answer this question and secondly to explore whether BON cells could be used for functional studies of heterologously expressed ORs.

BON cells were kindly provided by Kirk Ives (UTMB Galveston, USA). As the cell population appears heterogeneous in terms of morphology, we first serial diluted BON cells to isolate clones. RT-PCR was then carried out on RNAs from clones. RT-PCR products were subsequently cloned and sequenced. Clonal BON cells appear to express more than one OR and some ORs identified are expressed by several clones. To confirm the expression of several olfactory receptors by a single cell, single-cell RT-PCR was also performed.

Since BON cells endogenously express ORs, we expected they could also efficiently express heterologous ORs, which is still a challenge for most ORs in mammalian cells. Thus, we tried to heterologously express two human ORs (OR1G1 and OR17-40) in BON cells. We demonstrated that BON cells efficiently expressed these olfactory receptors at the plasma membrane level. Furthermore, we conducted calcium imaging and confirmed the activation of both receptors by their known ligands. While other ORs shoud be tested for functional expression by BON cells, these first results already suggest that BON cells constitute an interesting means to carry out functional studies of heterologously expressed ORs.

Endogenous expression of olfactory receptors by BON cells



RNAs preparation
DNase treatment
reverse transcription

Verification of absence of genomic DNA

nested PCR using pairs of degenerate primers corresponding to conserved regions of ORs

cloning and sequencing of PCR products

clone number	identified ORs
4	OR4F16
9	OR7E91P OR7E99P
10	OR13H1 OR7E99P
14	OR7A17 OR10Q1 OR7D2
19	OR1F1 OR13A1 OR7E38P OR2A20P (= OR2A9P)
23	OR2A1 (= OR2A42) OR6V1 OR1F1 OR13A1 OR2A20P (= OR2A9P)

pseudogenes

nested PCR using pairs of primers corresponding to variable regions of the previously identified ORs

sequencing of PCR products

Clone number	OR4F16	OR13H1	OR7A17	OR10Q1	OR7D2	OR2A1/2A42	OR6V1	OR1F1	OR13A1	OR7E38P	OR7E91P	OR7E99P	OR2A9P/2A20P
	identified ORs												
1	◉			◉	◉		◉	◉					◉
3	◉				◉			◉					◉
4	●	◉			◉								◉
8	◉			◉	◉			◉					◉
9				◉	◉			◉			●	●	◉
10		●		◉	◉			◉				●	
14	◉	◉	●	●	●			◉					
19	◉				◉			●	●	●			●
23	◉	◉				●	●	●	●				●

● and ◉ : ORs identified by nested PCR. ◉ : ORs identified using specific primers. pseudogenes

Various clones of BON cells express various groups of ORs, but a subset of ORs (OR7D2, OR1F1) is common to most of the clones.

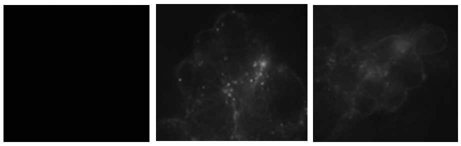
BON cells also express pseudogenes at the mRNA level.

Both OR7E38P and OR2A9P pseudogenes, as well as OR7A17, OR7D2 and OR2A1 are reported to be expressed in several tumors.

Clones of BON cells could have derived with time. We confirmed that single BON cells can indeed express several ORs using single-cell RT-PCR and specific primers targeting some of the previously identified ORs. We did not succeed in detecting all the receptors we were looking for. Nevertheless, we found that single cells of clone 14 co-express OR4F16 and OR10Q1.

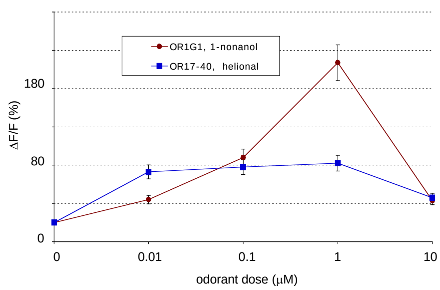
Functional heterologous expression of olfactory receptors by BON cells

BON cells were transiently transfected with mammalian expression vectors carrying the OR1G1 or OR17-40 receptor. Cells were observed 72h after transfection.



negative control OR1G1 OR17-40

BON cells heterologously express human OR1G1 and OR17-40 receptors. The receptors are fused to the c-myc epitope at their N-terminal end. They are revealed with an anti-cmyc antibody coupled to Cy3 on non permeabilized cells.



BON cells expressing OR1G1 or OR17-40 were loaded with fluo-4 and stimulated with various concentrations of cognate ligands of these ORs. They displayed a calcium response within seconds when the ligand of the expressed OR was applied, while mock-transfected BON cells did not.

Conclusion

Contrary to OSNs, BON cells were shown to endogenously co-express various functional ORs and pseudogenes at the mRNA level. While some receptors appear to be expressed by all cells, each clone of BON cells seems to express a specific panel of ORs.
In the olfactory apparatus, a combinatorial code is used to detect and discriminate odorants, involving several OSNs each expressing a single type of OR. In the case of BON cells, the variability in OR expression and the ability to co-express several ORs could be used for the detection of various odorants or other molecules brought by food intake into the gastro-intestinal tract.

Furthermore, we demonstrate that BON cells can express functional heterologous ORs. Thus, BON cells seem promising for contributing to the deorphanization of ORs and their functional study.