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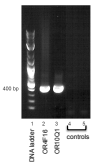
Olfactory receptors (ORs) are G protein-coupled receptors mainly expressed in the olfactory epithelium where they permit to detect and discriminate myriads of odorant molecules. OR are also known to be expressed in tissues other than olfaction-dedicated ones, with possible additional functions. For instance, ORs are reported to be overexpressed by tumor enterochromaffin cells and LNCaP prostate cancer cells, suggesting they could be tumor biomarkers. In this work, we first identified ORs present in tumor enterochromaffin cells (BON). Contrary to olfactory sensory neurons which express a single OR among several hundreds, we found that BON cells express various sets of ORs, probably contributing to an extensive detection of odorant molecules within the gastro-intestinal tract. Furthermore, some of the identified ORs have also been found in several tumors. We thus assumed an additional role for ORs in tumor progression. We demonstrated *in vitro* that ORs can promote cell invasiveness, taking advantage of heterologous expression of ORs in BON cells or using LNCaP prostate cancer cells that endogenously express an OR, the PSGR. We also showed that a PI3 kinase γ signaling pathway is involved in this process. Invasiveness potential being essential to metastasis emergence, we suspected that stimulation of ORs expressed by cancer cells *in vivo* could result in an increase of metastases occurrence. Using immunodeficient mice and subcutaneous implantation of LNCaP cells, we demonstrated that stimulation of these cells through PSGR odorant activation indeed promotes emergence of metastases.

Endogenous expression of ORs in enterochromaffin tumor cells (BON cell line)

OR present in clones of BON cells were identified by nested RT-PCR using degenerate primers targeting OR conserved regions, and primers specifically targeting OR identified with the degenerate primers.

Clone	OR4F16	OR13H1	OR7A17	OR10Q1	OR7D2	OR2A1	OR6V1	OR1F1	OR13A1
1	X			X	X		X	X	
3	X				X			X	
4	X	X			X				
8	X			X	X			X	
9				X	X			X	
10		X		X	X			X	
14	X	X	X	X	X			X	
19	X				X			X	X
23	X	X				X	X	X	X



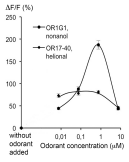
Example of nested PCR performed on RT products obtained from a single BON cell from clone 14, using two pairs of primers specifically targeting OR10Q1 or OR4F16. Each amplification product was about 400 bp long and verified by sequencing. Controls: without cell, with each pair of primers

Single BON cells co-express several ORs contrary to olfactory sensory neurons and different BON cell clones express various sets of ORs. Serotonin secretion of normal and neoplastic enterochromaffin (EC) cells is reported to be regulated by OR odorant activation. **Changes in the levels and variety of OR expression in EC cells could thus deregulate serotonin release and induce pathological disorders.**

Several ORs identified in BON cells (OR7A17, OR7D2 and OR2A1) are reported in other tumors. Other ORs are found overexpressed in EC tumor cells and in LNCaP prostate tumor cells. ➔ **The expression of some ORs in tumor cells could be part of their tumor phenotype.**

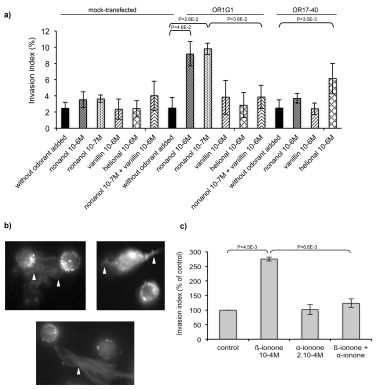
Functional heterologous expression of ORs in BON cells

Endogenous ORs identified in BON cells are orphan ➔ **heterologous expression of ORs with known ligands in BON cells to study their role in tumor cell invasiveness**



BON cells were transiently transfected to express OR1G1 or OR17-40 receptors. 72h later, cells were loaded with fluo-4 and stimulated with the respective odorant ligands of the transfected ORs. Calcium responses are expressed as the mean fluorescence variation ΔF/F (%). Mock-transfected cells did not respond to 1-nonanol nor helional.

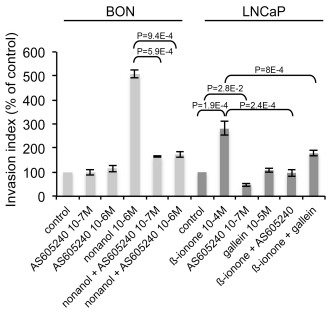
Odorant stimulation of ORs expressed in tumor cells promotes their invasiveness



(a) BON cells were transiently transfected to express OR1G1 or OR17-40 receptors or mock-transfected. Cells were seeded onto collagen type I gels and stimulated by the respective odorant ligands of OR1G1 and OR17-40 receptors (nonanol : OR1G1 agonist, vanillin : OR1G1 antagonist, helional : OR17-40 agonist). Invasion index (percentage of invasive cells) was determined 24 hours later. **(b)** F-actin cytoskeleton of BON cells in collagen gels was revealed by rhodamine-conjugated phalloidin. Arrows indicate invasive extensions. **(c)** LNCaP prostate cancer cells expressing an endogenous OR, the PSGR (Prostate Specific G protein-coupled Receptor), were seeded onto collagen type I gels and stimulated by PSGR ligands (β-ionone : agonist, α-ionone : antagonist). Standard deviation of the control was 13,42%.

Odorant stimulation of ORs expressed in each tumor cell line (BON or LNCaP) increases their invasiveness in collagen gels and this is counteracted by OR odorant antagonists

The promotion of tumor cell invasiveness by OR involves a PI3 kinase γ dependent signaling pathway

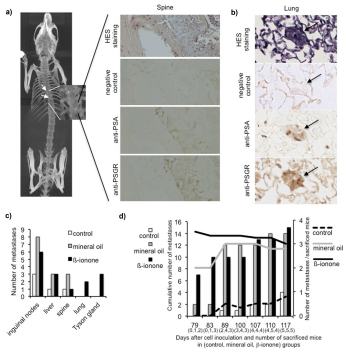


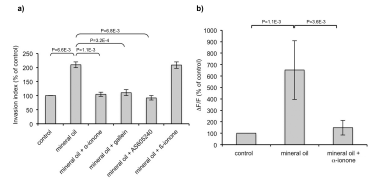
BON cells heterologously expressing the OR1G1 receptor were seeded onto collagen type I gels and stimulated by nonanol (OR1G1 agonist) in the presence of a specific PI3Kγ inhibitor (AS605240). LNCaP cells endogenously expressing the PSGR were seeded onto collagen type I gels and stimulated by β-ionone (PSGR agonist) in the presence AS605240 or gallein (inhibitor of βγ subunits of G proteins). Invasion index was determined 24 hours later and relative to that of control cells (without odorant, nor AS605240 nor gallein). Standard deviation of the control was 4,74% for the control BON cells and 13,86% for the control LNCaP cells.

PI3kγ inhibition by a specific inhibitor or through inhibition of βγ subunits of G proteins strongly reduces tumor cell invasiveness induced by OR odorant stimulation. Basal LNCaP cell invasiveness (without odorant stimulation) is also reduced by PI3Kγ inhibition.

➔ **An interplay between ORs, G proteins and PI3Kγ in tumor cells would promote their invasive phenotype.**

Stimulation of LNCaP prostate cancer cells by the PSGR agonist, β-ionone, promotes their ability to generate metastases *in vivo*





(a) LNCaP cells were seeded onto collagen type I gels and were stimulated by mineral oil or mineral oil mixed with β-ionone, α-ionone, AS605240 or gallein. Invasion index was determined 24 hours later and relative to that of control. Standard deviation of the control was 3,67%. (b) LNCaP cells were loaded with fluo-4 and stimulated with mineral oil or a mixture of mineral oil and α-ionone. Calcium responses are expressed as the mean fluorescence variation ΔF/F relative to that of control. Standard deviation of the control was 15,96%.

LNCaP cells were inoculated subcutaneously in immunodeficient castrated mice and mice skin was daily brushed with mineral oil or mineral oil containing β-ionone. (a) Example of mouse bearing metastases in the spine, observed by microcomputed tomography and confirmed by HES staining and immuno-histology using anti-PSA (prostate specific antigen) and anti-PSGR antibodies. (b) Example of metastasis found in a lung and characterized by HES staining and immuno-histology using anti-PSA and anti-PSGR antibodies. (c) Number of metastases in various tissues for each mice group (white: untreated control mice, gray: mice treated with mineral oil, black: mice treated with β-ionone in mineral oil). (d) Cumulative number of metastases, regardless of their location, as a function of the time elapsed between LNCaP cells inoculation and mice sacrifice. The number of sacrificed mice is indicated in brackets for each time point and each mice group. The curves report the ratios of the number of metastases over the number of sacrificed mice as a function of the time.

✓ Increased number of metastases in mineral oil and β-ionone treated mice
✓ Metastasis spreading in more diverse tissues only in β-ionone treated mice
✓ A component of mineral oil promotes LNCaP cell invasiveness and their ability to generate metastases through PSGR activation

CONCLUSION

Odorant stimulation of ORs expressed in tumor cells promotes their invasiveness *in vitro*, thus resulting in an increased ability to generate metastases *in vivo*, notably in the case of protaste tumor cells that endogenously express the PSGR. This OR can be activated in humans by steroid hormones, but since β-ionone is present in various products of our close environment (cosmetics, food and beverages), this exogenous odorant molecule activating the PSGR might be found in the body.

Hence, the identification of odorant molecules with the potency to activate ORs involved in tumor progression should permit to adopt prevention measures.

Moreover, identifying odorant antagonists inactivating ORs specifically expressed in tumor cells could allow to specifically target the tumors, limiting side effects.

In addition, future studies about the crosstalk between PI3K and OR signaling pathways should highlight effectors involved in cell invasion and metastasis emergence.

These results and their perspectives could thus lead to disruptive innovation in the treatment of cancers involving ORs.