Research Highlight

The Olfactory Receptor Pseudo-pseudogene: A Potential Therapeutic Target in Human Diseases*

CHEN Zhe, HUANG Zhen#, and CHEN Lin Xi#

Recently, Prieto-Godino et al.[1] found that the olfactory receptor 75a (*Ir75a*) gene is a functional pseudo-pseudogene in *Drosophila sechellia*. For a long time, Ir75a has been regarded as an acetic acid receptor that detects acetic acid and induces obvious olfactory responses in olfactory sensory neurons (OSNs)[2]. Nonetheless, Prieto-Godino et al. confirmed that Ir75a lost its sensitivity to acetic acid in *D. sechellia*. Thus, the *D. sechellia Ir75a* gene is generally recognized as a pseudogene in OSNs. Nevertheless, the *D. sechellia Ir75a* gene is not a simple pseudogene. Prieto-Godino et al. found that *D. sechellia* Ir75a is sensitive to propionic, butyric, and 2-oxopentanoic acids. Therefore, the *D. sechellia Ir75a* gene encodes a functional olfactory receptor (OR) that induces different olfactory responses.

The above results suggest that the *OR* gene should not be simply regarded as an olfactory pseudogene. Numerous studies indicate that the *OR* gene family is the largest gene family in human and mammalian genomes. There are approximately 1,000 *OR* genes identified in humans, whereas only 40% of *OR* genes have an intact coding region and are putatively functional[3]. Most of *OR* gene families are recognized as nonfunctional pseudogenes. Research has revealed, however, that many *OR* pseudogene fragments are translated, thus playing an important role in human cells[4]. Therefore, the *OR* gene is not a plain pseudogene, although the function of the *OR* pseudogene-derived proteins is unclear. Moreover, *OR* genes encode the largest subfamily of G protein-coupled receptors[5], and *OR*s are abundantly expressed in OSNs. *OR*s are activated by odorant ligands such as short-chain fatty acids, lactate, aldehydes, ketones, phenols, and alcohols. The activation of *OR*s can produce obvious smell perception by increasing intracellular Ca^{2+} in an OSN.

What are the reasons for the acquisition of a function by *D. sechellia* Ir75a? The read-through of a premature termination codon (PTC) permits the translation of *D. sechellia* Ir75a and changes the protein. Some studies show a homozygous mutation CAC to AA with an out-of-frame 1-bp deletion, which can lead to the stop codon read-through in the NADH-cytochrome b5 reductase gene[6]. In the *D. sechellia Ir75a* pseudogene, the C640T (CAA→TAA) nucleotide substitution in the open reading frame creates a PTC in exon 4, and this PTC permits read-through. The Ir75a PTC exists only in all the *D. sechellia* strains but not in any *D. melanogaster* or *D. simulans* strain. Read-through of the PTC allows for translation of the downstream sequence, leading to the extensions of the C terminus in *D. sechellia* Ir75a. Moreover, *D. sechellia Ir75a* gene read-through is detected only in diverse neuronal classes but not in non-neuronal cells. The neuron-specific read-through may be caused by an enriched tRNA in neurons. A tRNA can effectively recognize the Ir75a PTC and enables insertion of an amino acid instead of translation termination in *D. sechellia*.

The structure and function of the Ir75a protein are altered by the mutation and read-through in *D. sechellia*. Abundant studies suggest that a frame shift mutation triggers an alteration of hydrophobicity in the carboxyl-terminal portion, thereby resulting in disturbances of the structure and function of the protein[6]. In *D. sechellia* Ir75a, some changes occur at three mutation-prone ligand-binding positions including T289S, Q536K, and F538L. Moreover, *D. melanogaster* Ir75a can yield similar olfactory responses to propionic, butyric, and 2-oxopentanoic acids when mutated at these three positions. To some degree, these three positions can determine the sensitivity of Ir75a to different acids. Additionally, compared to *D. simulans* and *D. melanogaster* Ir75a, the side chains of the residues are different in *D. sechellia* Ir75a. Overall, these mutation-prone ligand-binding positions and residues as determinants of olfactory specificity are essential for the different ligand response profiles in *OR*s.

doi: 10.3967/bes2018.022

*This research is funded by grants from the National Natural Science Foundation of China [81470434, 81503074, 81670265]; Hunan Province Cooperative Innovation Center for Molecular Target New Drugs Study (Hunan Provincial Education Department document) [Approval number: 2014-405].

Institute of Pharmacy and Pharmacology, University of South China, Hengyang 421001, Hunan, China

Pseudo-pseudogenes are a widespread phenomenon in various species. Prieto-Godino et al. also found other *OR* pseudo-pseudogenes such as *Raleigh707 Ir75b*, *Raleigh441 Ir31*, and *Tasmanian09 Ir31*[1]. A nucleotide substitution results in a PTC of Ir75b and Ir31a. Moreover, Ir75b and Ir31a produce different olfactory responses to acids as compared with the *D. melanogaster* controls. These results suggest that *OR* pseudo-pseudogenes are a common phenomenon in *Drosophila*. *OR* pseudo-pseudogenes are not restricted to a particular species or a specific receptor repertoire. Furthermore, pseudo-pseudogenes are recognized as a result of natural selection, e.g., *via* food, habitat, or spouse. In the process of evolution, mutations and read-through of *OR* genes have produced a functional *OR* and ultimately ensured different olfactory responses.

A pseudo-pseudogene determines the population size of some species. In the process of evolution, a gene in some species is altered for adaptation to a change in the environment or food. On Seychelle islands, the ripe fruit of *Morinda citrifolia* is almost exclusive food for *D. sechellia*. Acetic acid is detectable at trace levels in the *Morinda* fruit. Hence, a nucleotide substitution and formation of a PTC happened in the *D. sechellia Ir75a* gene in order to adapt to the environment of Seychelle islands. The read-through of the PTC enables emergence of a function that allows for a low effective population size of *D. sechellia* among *Drosophila* species. Moreover, the odor-tuning properties of *D. sechellia* Ir75a have evolved, leading to a loss of the sensitivity to acetic acid and an increase in sensitivity to other acids. Therefore, the low effective population size of *D. sechellia* has persisted in *Drosophila* species on Seychelle islands.

The phenomenon of pseudo-pseudogenes may be involved in human diseases. Pseudogenes are still considered nonfunctional DNA relics in the human genome. Only lately was it well demonstrated that pseudogenes have widespread biological functions. Additionally, some pseudogenes may be a pseudo-pseudogene, which possibly play an important role in human disease. Research indicates that long noncoding RNAs transcribed from a pseudogene as crucial regulatory molecules are involved in human diseases such as cancer[7]. Besides, some studies show that PTC read-through is known only in viruses[8]. Human diseases can be associated with alleles that have low read-through rates[9]. Moreover, *OR*s are not only expressed in diverse olfactory neuronal classes but also ectopically expressed in human nonolfactory tissues or organs such as the heart, sperm, and skin cells as well as cancer tissues[10]. Ectopic *OR*s even perform diverse functions in nonolfactory tissues or organs[11]. Thus, *OR* pseudo-pseudogenes potentially generate variants by read-through in humans, and this mechanism may participate in the functional divergence of *OR*s and the development of a disease. Studies indicate that a novel mutation can occur in the NADH-cytochrome b5 reductase gene in type II recessive congenital methemoglobinemia[6]. Moreover, a frameshift with translational read-through of the natural stop codon is induced in the NADH cytochrome b5 reductase gene owing to a mutation and may lead to the development of type II recessive congenital methemoglobinemia. Therefore, targeting of the read-through induced by a mutation may be a novel treatment of type II recessive congenital methemoglobinemia. Identification of this read-through enables researchers to determine the molecular mechanism of action of DNA mutations in diseases. Thus, pseudo-pseudogene *Ir75a* is a functional DNA sequence in *D. sechellia* and is translated *via* PTC read-through (Figure 1). The

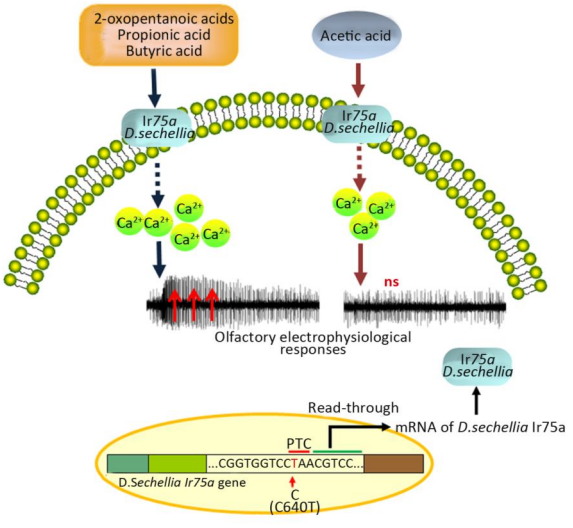

Figure 1. *D. sechellia* Ir75a is a functional olfactory receptor. A C640T (CAA→TAA) nucleotide substitution occurs in the *D. sechellia Ir75a* gene leading to the formation of a PTC. Read-through of this PTC allows for translation of *D. sechellia* Ir75a mRNA and the production of a functional olfactory receptor. This way, *D. sechellia* Ir75a has acquired greater sensitivity to propionic, butyric, and 2-oxopentanoic acids.

function of this pseudo-pseudogene requires more research on human diseases.

Conflict of Interest This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee. The authors declare no conflicts of interest.

#Correspondence should be addressed to CHEN Lin Xi or HUANG Zhen, Tel/Fax: 86-734-8683928, E-mail: lxchen6@126.com (CLX), huangzhenfy@126.com (HZ)

Biographical note of the first author: CHEN Zhe, female, born in 1990, MD, majoring in cardiovascular pharmacology.

Received: July 26, 2017;

Accepted: December 12, 2017

REFERENCES

1. Prieto-Godino LL, Rytz R, Bargeton B, et al. Olfactory receptor pseudo-pseudogenes. Nature, 2016; 539, 93-7.
2. Silbering AF, Rytz R, Grosjean Y, et al. Complementary function and integrated wiring of the evolutionarily distinct Drosophila olfactory subsystems. J Neurosci, 2011; 31, 13357-75.
3. Gilad Y, Bustamante CD, Lancet D, et al. Natural selection on the olfactory receptor gene family in humans and chimpanzees. Am J Hum Genet, 2003; 73, 489-501.
4. Ji Z, Song R, Regev A, et al. Many lncRNAs, 5'UTRs, and pseudogenes are translated and some are likely to express functional proteins. eLife, 2015; 4, e08890.
5. Fleischer J, Breer H, Strotmann J. Mammalian olfactory receptors. Front Cell Neurosci, 2009; 3, 9.
6. Leroux A, Leturcq F, Deburgrave N, et al. Prenatal diagnosis of recessive congenital methaemoglobinaemia type II: novel mutation in the NADH-cytochrome b5 reductase gene leading to stop codon read-through. Eur J Haematol, 2005; 74, 389-95.
7. Grandér D, Johnsson P. Pseudogene-Expressed RNAs: Emerging Roles in Gene Regulation and Disease. Current Topics in Microbiology and Immunology, 2016; 394, 111-26.
8. Bidou L, Rousset JP, Namy O. Translational errors: from yeast to new therapeutic targets. FEMS Yeast Res, 2010; 10, 1070-82.
9. Keeling KM, Xue X, Gunn G, et al. Therapeutics based on stop codon readthrough. Annu Rev Genomics Hum Genet, 2014; 15, 371-94.
10. Manteniotis S, Wojcik S, Gothert JR, et al. Deorphanization and characterization of the ectopically expressed olfactory receptor OR51B5 in myelogenous leukemia cells. Cell Death Discov, 2016; 2, 16010.
11. Chang AJ, Ortega FE, Riegler J, et al. Oxygen regulation of breathing through an olfactory receptor activated by lactate. Nature, 2015; 527, 240-4.