Activation of GPR15 and its involvement in the biological effects of smoking

Sulev Kõks[1,2], Gea Kõks[1]

[1]Department of Pathophysiology, University of Tartu, Tartu 50411, Estonia;

[2]Department of Reproductive Biology, Estonian University of Life Sciences, Tartu, Estonia

Address for Correspondence:
Sulev Kõks
19 Ravila Street, Tartu 50411, Estonia
Phone: +372 7374 371
Fax: +372 7374 372
e-mail: sulev.koks@ut.ee

Abstract

Smoking is one of the most significant modifiable environmental risk factors for many diseases. Smoking causes excessive mortality worldwide. Despite the decades long research, there has not been clear understanding of what is the molecular mechanism that makes smoking harmful to the health. Some recent studies have found that smoking influences most significantly the expression and methylation of GPR15. GPR15 is an orphan receptor that is involved in the regulation of the innate immunity and the T-cell trafficking in the intestinal epithelium. Further studies have confirmed that GPR15 is very strongly involved in smoking and smoking-induced molecular changes. Therefore, the altered expression and epigenetic regulation of GPR15 could have a significant role in the health impact of smoking.

Keywords:

tobacco smoking, GPR15, transcriptome, epigenetics, immunology, genomics

Impact statement

The review describes an orphan receptor GPR15 that has recently been found to be influenced by smoking. This makes GPR15 very sensitive and adequate biomarker for smoking and smoking studies. Also, activation of GPR15 by smoking could help to explain its effects on health.

Introduction

G-protein-coupled receptor 15 (GPR15) was found during an attempt to identify novel opioid and peptide receptors sharing the similarities with other G-protein coupled receptors (1). In this initial study, the gene for GPR15 was localised to the chromosome 3 region 3q11.2-q13.1 (Figure 1). In human genome, GPR15 gene is in between the claudin domain containing 1 (CLDND1) and coproporphyrinogen oxidase (CPOX) genes. The amino acid sequence of GPR15 shares identity with the angiotensin II AT1 and AT2 receptors, the interleukin 8b receptor and the orphan receptors GPR1 and AGTL1 (1). GPR15 is an orphan chemokine receptor whose natural ligand is not identified. Another orphan receptor, GPR25, was identified later to have the highest identity to GPR15 (2). GPR15 gene is an intronless single-exon gene with 1252 nucleotides and encodes the 360 amino acid protein (1). The protein is expressed on the cell membrane and it is considered a chemokine receptor (also designated as BOB/GPR15) and it functions as a co-receptor for the human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2) (3, 4). GPR15 is a heterotrimeric G-protein-coupled receptor, that controls the specific homing of FOXP3-positive regulatory T-cells (T-regs) (5). Accumulating evidence indicate that GPR15 regulates the innate immunity and it is involved in pathogenesis of diverse diseases like Crohn's disease and rheumatoid arthritis (6-8). Several recent studies have found that smoking significantly increases expression of GPR15 making it one of the most interesting biomarker of smoking. In the present paper we review the knowledge about functions of GPR15 receptor and its potential impact in human diseases caused by smoking.

G protein-coupled receptor 15 (GPR15)

Early studies found that GPR15 is co-receptor for the CD4-dependent simian immunodeficiency virus (SIV), HIV-1 and HIV-2 (9, 10). In an expression cloning study, two new chemokine receptors for SIV and HIV were identified (11). One was named "Bonzo" and the second was designated BOB (for brother of Bonzo). Sequence analysis indicated that both molecules were members of the G-protein-coupled receptor family (11). In this original research report, BOB was identified as previously cloned GPR15, but for Bonzo no identity was found. Another study identified STRL33 as a co-factor for macrophage-tropic and T cell line-tropic HIV-1 (12). Bonzo was recognized as STRL33 in the original study of its discovery (11). Eventually Bozo/STRL33 was identified as C-X-C motif chemokine receptor 6 (CXCR6) (13). Therefore, GPR15 is a chemokine receptor with high similarity to other members of the chemokine receptor family. GPR15 lacks the third extracellular loop that is thought to be disulphide linked. Although the sequence of GPR15 is divergent from the other identified primate immunodeficiency virus co-receptors, its amino terminus contains three tyrosines which align with similarly positioned tyrosines in CCR5 (10). These tyrosines are necessary for the efficient SIV and HIV-1 entry. Further studies were performed to analyse the importance of GPR15 in the virus entry and replication. Compared to the Bonzo/CXCR6, GPR15 was more frequently used as a co-factor for the HIV-1 envelop mediated entry (14). Despite the lower efficiency of GPR15 usage, it mediated majority of the HIV-1 entries (14). This indicates that GPR15 is used for productive infection and HIV-1 transmission. In case of HIV-2 infection, the recruitment of GPR15 is not that important and it plays minor role as a co-factor for the pathogenicity of the HIV-2 infections (15).

Expression of GPR15 and its regulation

Initial study found GPR15 to be expressed in lymphatic tissues and in colon (11). Similar results are evident also in the GTEx portal (http://www.gtexportal.org/). RNAseq data from various human tissue samples indicate that the highest expression of GPR15 is in the transformed lymphocytes, median RPKM is 99. It is also expressed in a colon (RPKM 3.6) and in the terminal part of small intestine (RPKM 5.3). According to this database, the expression of GPR15 is very low in the other tissues. In the Figure 1, small overview of the expression profile of GPR15 is given. However, the comprehensive analysis of the expressional pattern of GPR15 is missing and GPR15 expression has been detected also in the bladder, heart and skin (16, 17). Couple of studies have addressed the question of GPR15 tissue specificity and its involvement in the pathogenesis of diseases. In one study immunoblots for several human tissues were performed (17). Clear expression of GPR15 protein was detected in colonic mucosa, lymph node, prostate, testis and liver. No bands were detected in the brain, placenta, lung, uterus, heart, pancreas or skeletal muscle (17). Moreover, the same study indicated that HIV-1 envelope surface protein gp120 is able to induce GPR15 activation and this is considered to be plausible mechanism for HIV-induced enteropathy (17). This allowed authors to propose the GPR15-dependent virotoxin model of HIV-1 enteropathy (18).

In another study, comprehensive analysis of the expression of genes for different chemokine receptors in human and simian astrocytes was performed using semiquantitative RT-PCR technology(19). Authors found robust and significant expression of the GPR15 in human fetal, human adult and simian astrocytes. Moreover, authors found that stimulation with TNFα and IL-1β significantly

increased the expression of GPR15 (19). This finding suggests that the activation of GPR15 could also be responsible for the brain pathology caused by HIV infection or for the HIV dissemination in the brain parenchyma (19).

There are studies that indicate involvement of GPR15 in the apoptosis of different cells. The higher expression of GPR15 in the surface of polymorphonuclear neutrophils (PMN) has been found to account for the increased death induced by the SIV infection in macaques (20). The SIV infection induced death of the PMNs by the mitochondrial membrane permeabilization. This damage occurs independently in the Bax and Bak. Therefore SIV induced death is mediated via GPR15 without involving the major mechanism inducing the loss of the mitochondrial membrane potential (20). The same study used specific antibodies and found the engagement of GPR15 induced death of PMNs in a manner similar to the virus itself (20). This experimental finding suggests that GPR15 is not only involved in the SIV entry, but participates significantly (with unknown mechanism) in the cell death caused by the immunodeficiency virus (20).

In addition to the induction of apoptosis in PMNs, infection with SIV or HIV-1 causes enteropathy in early stages of infection. Gut epithelial cell apoptosis was found to coincide with the interaction between virus and GPR15 (21). Only background levels of viral RNA were detectable before and at the onset of the gut infection. Similarly, GPR15 expression was mainly detectable in the basal surfaces of epithelium. On the contrary, at the peak of apoptosis, significantly increased virion binding to the basal surfaces of the gut epithelium was observed. Increased virion binding was accompanied by the transcytosis and shedding of GPR15 into intestinal lumen (21). This increase was reverse for the

28th day after infection. Authors speculated that the peak apoptosis is the result of virotoxic effects that virion pg120 has on gut epithelial cells shown in previous *in vitro* studies (17, 18). The finding in gut epithelial cells is in a very good line with the data from the previously discussed PMN study that indicated GPR15 involvement in apoptosis (20). Taken together all these data suggest that activation of GPR15 can induce apoptosis in the immune and epithelial cells.

The expression of GPR15 has been identified to be induced by infection in a variety of human immune cells like $CD4^+$, $CD8^+$ and $CD19^+$ (22). Viral components can induce the expression of GPR15, but this is not necessary for the SIV or HIV infection of $CD4^+$ cells (22). Expression of GPR15 in the central memory T cells expanded the potential role of GPR15 from the HIV/SIV target cell population to a bigger part of another $CD4^+$ cell population. This finding initiated great interest to find what other factors in addition to the virus components are able to induce the expression of GPR15. Indeed, it was soon found that GPR15 expression is up regulated by the Toll-receptor 3 (TLR3) signalling via TIR-domain-containing adapter-inducing interferon-β (TRIF) (23). This finding is in a good line with earlier studies, where phosphoinositide-3 kinase (PI3K) activation was shown to induce GPR15 surface expression (24). At the same time, the PI3K can be activated via TLR3 signaling pathway and therefore the regulation of GPR15 expression by TLR3 was suggested (25). Up-regulation of GPR15 was most prominent in gut homing $CD4^+$ T cells and it is highly expressed on intestinal $CD4^+$ T cells. Taking into account that GPR15 has also role in apoptosis, it was proposed that it has importance in gut inflammation and in the destruction of the intestinal epithelium (23).

GPR15 and its involvement in diseases

In addition to the role of HIV and SIV infection, GPR15 has a more general role in the regulation of innate immunity and regulation of the homing of the T-cell in gut epithelium (5, 8). The homing regulation is most clearly indicated in case of a large intestine where regulation of $FOXP3^+$ regulatory T-cells by GPR15 was found. The GPR15 expression in a large intestine can be modified by gut microbiota and TGFβ1 (5). GPR15 is specifically responsive for the large intestine homing. When $GPR15^+$ cells and control cell were mixed at 1:1 ratio and then transferred into C57BL/6 mice, all tissues exhibited 1:1 ratio except large intestine where around 10-fold enrichment for GPR15 was found (5). In the same report it was described that GPR15 knockout (KO) mice had increased proportion of IFN-γ and IL-17A producing cells in the lamina propria of the large intestine indicating inflammation. Subsequent infection of mice with the *Citrobacter rodentium* revealed that most mice lacking GPR15 suffered from severe weight loss and died due to the infection (5). Wild-type mice survived and resolved the inflammation. GPR15 KO mice exhibited increased inflammation, tissue damage and inflammatory cytokine expression. Also, the number of T_{reg} cells was reduced in the GPR15 KO mice (5). Moreover, GPR15 is able to suppress noninfectious inflammation. Transfer of T_{regs} from wild-type mice, but not from the GPR15-deficient mice, reduced colitis severity and tissue damage induced by CD40 antibody. These experiments indicated that GPR15 is critical to prevent pathological inflammation in the large intestine during colitis and is most likely mediated by the regulation of the homing of T_{reg} (5).

The intestinal mucosa is the largest body surface exposed to the environment and microbial diversity. In addition to the inflammatory signals, normal gut

microbiota can have an impact on the GPR15 expression and therefore, GPR15 could have a role in the normal intestinal balance in micorbiome. Indeed, the treatment of mice with broad-spectrum antibiotics decreased the expression of GPR15 (5). Immune responses between the small bowel and colon have many common features, but there are also striking differences in their mechanisms of immune regulation. For instance, retinoic acid (RA) signaling via RA nuclear receptors plays a key role in immune homeostasis in the small bowel (26). Recent work indicates that RA is required for establishing immune tolerance to dietary antigens in the upper intestinal tract by inducing gut-tropic T_{reg}. On the other hand, microbiota-specific T_{reg} in the colon can be regulated by short-chain fatty acids (SCFA). Moreover, for homing T_{reg} utilize GPR15, which is up regulated by SCFA (5, 26). Thus, dietary SCFAs are playing key roles in the mechanisms governing intestinal tolerance to dietary antigens in the colon by recruiting GPR15 receptors (26).

In addition to the inflammatory and dietary signals, the GPR15 has been found to be up-regulated in response to the dioxin-stimulation in several different cell lines (27). This activation was rapid and it is the primary response to the dioxin because it was evident also in the presence of cycloheximide, the protein synthesis inhibitor (27). This indicates that GPR15 can respond to more common environmental signals.

While the expression of GPR15 was found to be specific for the T_{reg} cells, another study found GPR15 also in the T_H17 and T_H1 effector cells in mouse. Expression of GPR15 in effector T cells makes it very important regulator for the development of colitis. Indeed, GPR15 was found to be required in the colitis models that depend on the trafficking of the T_H17 and T_H1 cells (8, 28). In

humans, GPR15 is also expressed in pathogenic T_H2 cells in case of ulcerative colitis and this finding is in striking contrast with mouse data (8). In the study focusing on the role of GPR15 in the colitis, its role in the T cell trafficking and specifically in the recruitment of effector T cells was described. Namely, T cells from colons of individuals with ulcerative colitis have a much higher proportion of $GPR15^+$ cells and these cells also express IL-5 and IL-13 (8).

Differential expression of GPR15 between the mice and humans was found to be caused by differences in the regulatory sequences. Namely, in humans, GPR15 is regulated with the GATA3 enhancer in TH_2 cells and with FOXP3 in T_{reg} cells (8, 28). In TH_2 cell of mice, the GATA3 enhancer sequence is altered that makes it inefficient, but in T_{reg} cells, FOXP3 can stimulate the expression of GPR15 (8, 28). These differences can explain why GPR15 regulates more T_{reg} cells in mice and regulates T_{eff} cells (6) in humans. Taken together, GPR15 is involved in the trafficking of T cells in the colon, but additional detailed information is needed.

While most of the studies have described the homing of T cells in the intestine and the role of GPR15 in the intestinal inflammation, other studies have found GPR15 activity in other tissues. One earlier study found GPR15 expression in synovial membrane specific to the rheumatoid arthritis (29). This is a new finding and has not been challenged by any other similar study.

In another study the GPR15 was shown to be involved in the homing of dendritic epidermal T cells (DETC) (16). GPR15 is highly expressed in fetal thymus DETC precursors and on recently recruited DETCs. It was postulated that GPR15 mediates the earliest seeding of the epidermis. However, it is not clear if GPR15 participates in the cutaneous T-cell homing of the adult. Taken together, GPR15 seems to have a role in the homing of resident immune cells in the epithelial

tissues, both in the intestinal epithelium and in the skin. As a result, GPR15 is very likely to be involved in the inflammation of these target tissues, colitis and dermatitis. For colitis, there is convincing evidence for GPR15 involvement, for dermatitis and arthritis, further studies are required.

Smoking-induced molecular changes

Tobacco smoking is a single major cause of premature death worldwide (30, 31). Despite substantial reduction in the use of tobacco, smoking still causes globally more deaths than diseases like tuberculosis, HIV and malaria together, making it the largest preventable health risk factor (32, 33). The use of tobacco is legally allowed and therefore the prevalence of smoking behaviour is still very high. While in the developed countries tobacco use is reduced and restricted, in the developing countries the tobacco epidemic is still in a growing phase (30, 34). It has been estimated that tobacco smoking causes globally 6 million deaths in a year (35). 80% of these deaths are premature and hit the population with lower income (34). All this makes tobacco smoking the largest single avoidable cause of mortality. Reducing the prevalence of smoking increases the health of general population and avoids premature disability and death. Quitting of smoking and supporting to stop smoking are the easiest tools to improve the quality of life in population (36, 37). The molecular mechanisms of how smoking causes harm to the health have been extensively studied and a large amount of data have been produced. The effect of smoking on health is very complex and several different long-lasting molecular changes occur. GPR15 has recently been identified to be one of the most significantly and reproducibly induced alteration by smoking.

GPR15 and smoking

While it's clear that smoking increases mortality, the association between smoking and mortality is different across specific causes of death. Cancers, chronic obstructive diseases of respiratory system and cardiovascular diseases are the most commonly referred smoking-induced causes of death. The impact of smoking to these causes of death can persist for prolonged periods after smoking cessation. The involvement of epigenetic reprogramming in long term smoking impact has been proposed. Identification of the molecular pathways that contribute to the biological influence and disease-causing effects of smoking may offer opportunities for diagnostics and therapeutics. Therefore, recently several genomic analyses have been performed to find molecular targets responsible for smoking induced reprogramming. Most of the studies used methylation analysis, few studies have analysed genome-wide transcriptome changes.

The first study that found hypomethylation of the GPR15 locus was performed on a cross-sectional cohort and altogether 1454 people were analysed (38). The study identified 15 methylated sites significantly associated with the current smoking, 2 sites with cumulative smoke exposure, 3 sites were associated with the time since quitting of smoking. Two loci were significantly changed for all three conditions, factor II receptor-like 3 (F2RL3) and G-protein-coupled receptor 15 (GPR15) (38). Another study with African Americans confirmed this initial finding (39). They used 972 persons in discovery sample and 239 persons in replication sample. Differential methylation of GPR15 locus by smoking was independently found in another study (40). Methylation analysis of the 111 African American females identified two major loci to be differently methylated between smoker and non-smokers – aryl hydrocarbon receptor (AHRR) and

GPR15. This study also found an activation of immune response in smokers. According to this work smoking induces extensive effects on peripheral mononuclear cell DNA methylation and this is related to the molecular pathways of coagulation, CNS and immune function (40). They concluded very precisely, that smoking is important confounder and should be included in future diagnostic models in epidemiologic and clinical research to accurately understand diseases. In their next study, the same authors found that methylation of GPR15 locus that was dependent on the ethnicity and hypomethylation was found only in African Americans (41). However, several following studies have found the highly significant (with genome-wide significance) hypomethylation and increased expression of GPR15 in smokers (42-44).

Another study analyzed RNA expression form the whole blood together with methylation profile and found highly significant up-regulation of GPR15 expression in the blood that correlated with the hypomethylation of the GPR15 locus (45). These authors found that the methylation was reversible after smoking cessation. In addition, one more focused study analyzed the effect of smoking to the different cell subtypes in the blood (43, 44). Smoking clearly increased the expression of GPR15. The main cell population expressing GPR15 are $CD3^+$ cells (44). The authors also found that smoking increases the proportion of $GPR15^+$ cells among $CD3^+$ T cells, from 3.7% of non-smokers to 15.5% of smokers (44). The authors even suggest that the cutoff of 9% for GPR15 on the expressing cells can distinguish smokers from non-smokers with high sensitivity and high specificity. Thus, the tobacco smoke induced methylation changes at single CpG site are due to an increased proportion of

specialized cell subtypes rather than a direct effect of tobacco smoke on DNA methylation (44). Moreover, authors also show that *in vitro* stimulation of PBMCs with the cigarette smoke extract (CSE) did not increase the expression of GPR15 or the proportion of $GPR15^{+}$ cells. Therefore, CSE is not directly responsible for the hypomethylation of GPR15 locus (cg19859270) and there is no causative effect of smoking to the DNA methylation. By excluding the direct action, more complex cascade of tobacco-smoking-induced disturbance of tissue homeostasis was proposed (44).

One recent systematic review analyzed all the methylation studies performed in relation to smoking and found that three loci are the most consistently found to be differentially methylated – GPR15, AHRR and F2RL3 (46). While GPR15 hypomethylation correlates very well with the increased RNA expression, with AHRR only methylation changes have been described and no difference in gene expression has been found (42). Therefore, GPR15 is almost the only gene related to smoking and having a clear correlation of methylation and expression.

Conclusions

Altogether, GPR15 seems to be a very good biomarker for the studies of smoking. The cutoff of 9% for GPR15+ cells has been suggested and GPR15 is almost the only gene which expression correlates with biologically verified smoking status (exhaled carbon monoxide) (44, 47). GPR15 regulates immunity and our knowledge about its function is still very limited. As GPR15 is clearly involved in the biological effects of smoking (chronic inflammatory diseases) and the effect is not caused by direct action to GPR15, more studies on the functions of GPR15 and its relations to tobacco smoking are needed.

Authors Contribution Statement

Sulev Kõks planned the topic of the review and the structure of the review, writing of manuscript;

Gea Kõks performed literature search and analysis, writing of manuscript

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Figure legend

Figure 1. The overview of the genomic locus of GPR15 gene indicates single exon. Also several human mRNAs are shown, they all have similar structure. Barplot indicates gene expression profile in different tissue. It is evident that GPR15 has very specific expression pattern and is expressed in limited number of tissues.