Non-coding RNA Research xxx (2018) 1–12

Contents lists available at ScienceDirect

Non-coding RNA Research

journal homepage: http://www.keaipublishing.com/NCRNA

Crosstalk mechanisms between the WNT signaling pathway and long non-coding RNAs

Vasiliki Zarkou [a, b], Alexandros Galaras [a, c], Antonis Giakountis [a, d], Pantelis Hatzis [a, *]

[a] Biomedical Sciences Research Center 'Alexander Fleming', 16672 Vari, Greece
[b] School of Biology, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
[c] Department of Medicine, National and Kapodistrian University of Athens, 11527 Goudi, Greece
[d] Department of Biochemistry and Biotechnology, University of Thessaly, 41500 Larissa, Greece

ARTICLE INFO

Article history:
Received 27 January 2018
Received in revised form
5 April 2018
Accepted 5 April 2018
Available online xxx

Keywords:
WNT/β-catenin signaling
lncRNAs
Long non coding RNAs
Cancer

ABSTRACT

The WNT/β-catenin signaling pathway controls a plethora of biological processes throughout animal development and adult life. Because of its fundamental role during animal lifespan, the WNT pathway is subject to strict positive and negative multi-layered regulation, while its aberrant activity causes a wide range of pathologies, including cancer. At present, despite the inroads into the molecules involved in WNT-mediated transcriptional responses, the fine-tuning of WNT pathway activity and the totality of its target genes have not been fully elucidated. Over the past few years, long non-coding RNAs (lncRNAs), RNA transcripts longer that 200nt that do not code for proteins, have emerged as significant transcriptional regulators. Recent studies show that lncRNAs can modulate WNT pathway outcome by affecting gene expression through diversified mechanisms, from the transcriptional to post-translational level. In this review, we selectively discuss those lncRNA-mediated mechanisms we believe the most important to WNT pathway modulation.

1. Introduction

The WNT signaling cascade is highly conserved among species and controls a multitude of biological processes during animal development and life-cycles. Because of its central role in the maintenance of tissue homeostasis, the WNT pathway is tightly regulated at multiple levels, from the ligand-receptor interaction down to transcriptional and post-transcriptional levels; its aberrant activity has been implicated in a number of developmental disorders and diseases and, most prominently, in cancer [1]. Although the main molecular players of this pathway have been well characterized, aspects of its function, including the fine-tuning of its activity and the totality of its target genes remain incompletely understood. The recent discovery of long non-coding RNAs (lncRNAs) that are regulated by WNT and/or participate in WNT pathway modulation and outcome is particularly intriguing and has highlighted some of these gaps in our knowledge [2,3].

LncRNAs comprise a group of non-coding transcripts, arbitrarily defined as being longer than 200nt, that are transcribed from the human and other genomes but do not code for proteins [4,5]. Despite the lack of obvious protein-coding potential, functionality has been assigned to several lncRNAs. They have been shown to participate in many cellular processes such as gene imprinting [6], differentiation and development [7], antiviral immunity [8] and transcriptional responses [9,10]. Therefore, deregulation of lncRNA expression and function is implicated in the pathogenesis of several diseases, including cancer [2,11], metabolic [12,13], cardiovascular [14], neurodegenerative [15] and inflammatory pathologies [16,17].

In this review, we summarize our knowledge on the WNT signaling cascade both in normal and disease conditions. In addition, we provide a brief overview of the progress made in the lncRNA field, focusing mainly on lncRNA activities in modulating gene expression. Finally, we highlight the molecular mechanisms underlying the crosstalk between WNT/β-catenin signaling and lncRNAs, as well as the relevance of this interplay in different pathological conditions.

2. WNT/β-catenin signaling pathway

The WNT signaling cascade is divided into β-catenin dependent

* Corresponding author.
E-mail address: hatzis@fleming.gr (P. Hatzis).

https://doi.org/10.1016/j.ncrna.2018.04.001

(WNT/β-catenin) [1] and β-catenin-independent signaling branches (WNT/Planar Cell Polarity, WNT/Calcium and WNT/JNK pathways) [18]. The WNT/β-catenin signaling pathway, also known as the canonical WNT pathway, is better characterized and comprises signal transduction from the extracellular membrane to the nucleus through the accumulation of β-catenin protein [1]. In unstimulated cells, the cytoplasmic levels of β-catenin are maintained low by a destruction complex consisting of tumor suppressor proteins adenomatous polyposis coli (APC) and Axin2 and the kinases casein kinase1 (CK1) and glycogen synthase kinase 3β (GSK3β) [1]. The constitutively active CK1 and GSK3β kinases phosphorylate Axin-bound β-catenin at a series of Ser/Thr conserved residues [19] inducing the recruitment of the F-box-containing protein E3 ubiquitin ligase β-TrCP [20]. The subsequent ubiquitination of phospho-β-catenin triggers its proteasomal degradation [21] thereby abrogating its nuclear translocation. In the absence of nuclear β-catenin, its effector T cell Factor (TCF)/Lymphoid Enhancer Factor (LEF) transcription factors [22,23] interact with Groucho proteins, mediating transcriptional repression of WNT target genes [24,25]. Binding of WNT ligands to their cognate Frizzled (Fzd) receptors induces the formation of a heterodimeric complex consisting of Fzd and low density lipoprotein receptor-related protein 5/6 (LRP5/6) co-receptors [26,27]. This leads to a conformational change of the cytoplasmic tail of LRP5/6, making it accessible to phosphorylation by several protein kinases. As a result, the Axin2 protein is recruited to the cell membrane by interacting both with the phospho-tail of LRP5/6 and the Dishevelled (Dsh) protein, which binds to the cytoplasmic part of Fzd receptors, leading to inactivation of the destruction complex and stabilization of β-catenin [1,28,29]. Accumulated cytoplasmic β-catenin then migrates to the nucleus, where it engages context-dependent DNA-bound TCF/LEF transcription factors, converting them to transcriptional activators [1,29]. Aberrant WNT/β-catenin activation, due to mutations in components of the destruction complex or β-catenin itself, is a common hallmark of many cancers, resulting in the expression of WNT target genes with oncogenic functions [30].

WNT proteins are not the only ligands of Fzd/LRP receptors; there are several molecules competing with WNTs for binding. WNT antagonists of the Dickkopf (DKK) and the Sclerostin/SOST families block the WNT signaling cascade by binding to LRP5/6 and abrogating its subsequent dimerization with Fzd [31,32]. Similarly, secreted Frizzled-related proteins (sFRPs) bind and inactivate Fzd receptors. Other WNT inhibitors function by binding to and neutralizing WNTs. For example, WNT inhibitory proteins (WIF) bind WNTs, thereby preventing pathway activation [26]. Similarly, APCDD1, a membrane-bound glycoprotein, disrupts WNT signaling by binding both WNT ligands and LRP [33]. On the other hand, Norrin (NDP) and R-spondins (RSPO) function as WNT agonists by binding and activating Fzd receptors [1]. The Norrin protein interacts specifically with the Fzd-4/LRP5 complex and activates signal transmission into cells during retinal vascularization, while R-spondin 1-4 proteins interact with Lgr4/5/6 receptors and enhance signaling at low WNT levels [34–37]. Binding of R-spondins to Lgr4/5/6 receptors enhances WNT signaling by clearing the cell-surface transmembrane E3 ubiquitin ligases, zinc and ring finger 3 (ZNRF3) and its homologue, ring finger 43 (RNF43), from the plasma membrane. RNF43 and ZNRF3 ligases ubiquitinate the cytoplasmic tails of Fzd receptors, inducing their rapid endocytosis and lysosomal degradation, inhibiting the WNT pathway [37–39].

2.1. WNT/β-catenin signaling function and dysfunction

The WNT/β-catenin signaling cascade plays crucial roles in animal life by controlling various genetic programs during embryonic development and adult homeostasis [40]. Specifically, WNT, along with other signaling pathways, such as TGF-β, Hedgehog, Notch and Receptor Tyrosine kinases, participate in controlling cellular proliferation, differentiation, cell migration and apoptosis [40]. It is therefore not surprising that aberrant function of these pathways leads to severe developmental perturbations and lethality [40]. The same pathways also play significant roles in the maintenance of tissue homeostasis and regulate stem cell functions during adult life [40]. Stem cells possess the ability to self-renew, while also giving rise to the specialized cells maintaining tissue architecture and homeostasis [41]. The WNT/β-catenin pathway has been found to be required for the maintenance of many stem-cell types by controlling, among others, the expression of LGR5 and AXIN2, two stem cell specific genes [42,43]. Both Lgr5 and Axin2 have been used in lineage tracing experiments, increasing our knowledge about WNT-regulated adult stem cells in many organs and tissues [44–48]. A representative example for the dependence of stem-cell self-renewal on WNT/β-catenin signaling is the mammalian intestinal epithelium; genetic ablation of Tcf4 in mice results in epithelial breakdown due to the loss of intestinal stem cells [49]. In other examples, inhibition of WNT signaling by overexpression of DKK (WNT antagonist) eliminates hair follicles and disrupts the mammary gland by influencing the residing stem cells and their progenitors [44,50,51]. Similarly, in the hematopoietic system, overexpression of Axin (a negative regulator of the WNT pathway) decreases the number of transplantable stem cells [52]. On the other hand, activation of WNT signaling in the presence of WNT ligands or by using constitutively active forms of β-catenin leads to stem cell expansion in hematopoietic and hair follicle systems, respectively [53,54].

Given the importance of the WNT/β-catenin signaling cascade for adult stem cell biology, it is expected that mutations in its components are frequently observed in cancer, mainly in tissues that normally depend on WNT for self-renewal and repair [55]. One of the most well-established examples is colon cancer. Familiar adenomatous polyposis (FAP) is a hereditary cancer syndrome caused by germline mutations in the APC gene [56–58]. FAP patients carry heterozygous APC mutations, and loss of heterozygosity leads to the formation of polyps in adulthood. Additional mutations in oncogenes or tumor suppressor genes drive these polyps towards malignancy. In sporadic colorectal cancers, both APC alleles are frequently lost, resulting in β-catenin stabilization and constitutively activated WNT signaling that fuels cancer cell growth [59]. Inactivating mutations in other WNT pathway components such as Axin2 [60], β-catenin [61], RNF43 [62] and ZNRF3 [63] have also been reported to cause a variety of carcinomas in different tissues.

3. LncRNAs in the WNT signaling cascade

In recent years, lncRNAs have gained prominence as integral regulators of gene expression from the transcriptional to the post-transcriptional and translational levels; they have also been shown to respond to signaling molecules and to affect signal-dependent cell functions [64]. LncRNAs crosstalk with a variety of key signaling networks, such as WNT [65,66], Notch [67], TGFβ [68,69] and p53 [64], and affect many cellular pathways and biological processes, including oncogenic signaling. Recent advances in biomedical research have allowed the implementation of experimental and bioinformatic approaches to identify WNT-associated lncRNAs as both regulators and targets of the WNT/β-catenin signaling cascade.

3.1. The emergence of long non-coding RNAs

In 2007, the Encyclopedia of DNA elements (ENCODE) project, an

Please cite this article in press as: V. Zarkou, et al., Crosstalk mechanisms between the WNT signaling pathway and long non-coding RNAs, Non-coding RNA Research (2018), https://doi.org/10.1016/j.ncrna.2018.04.001