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Where Wnt is going: Revolutions in Wnt research

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Wnt proteins are important metazoan signaling molecules that help regulate stem cell maintenance, cell proliferation, and cell differentiation. Mutations in Wnt genes are associated with several hereditary diseases, including bone diseases and cancers. This microreview probes a few current studies that (a) revise and enhance our understanding of the Wnt signaling pathway, and (b) identifies novel targets for cancer treatment. Despite the progress, several features of Wnt signaling remain unknown. Accordingly, a few open questions related to Wnt protein/pathway functions conclude this microreview.

Introduction

Wnt proteins are important metazoan signaling molecules that help regulate stem cell maintenance, cell proliferation, and cell differentiation (Clevers and Nusse, 2012; Li et al., 2012). Research indicates that Wnt proteins are highly conserved in terms of evolution. For example, Wnt genes are found in sponges, sea anemones, worms, flies, frogs, mice, and humans. Because unicellular organisms do not have Wnt genes, but the first multicellular organisms do, e.g. sponges, Clevers and Nusse (2012) suggest that Wnt-signaling may have been key in the evolution of multicellular animals. Bazan et al. (2012) pointed out that although Wnt proteins have not been observed in amoebas and fungi, some of these organisms possess the same receptors that bind Wnt proteins in metazoans, which suggests an evolutionary relationship complementary to Clevers and Nusse's suggestion. A final evolutionary note is that several Wnt homologs are found among flies, frogs, and mammals. In fact, the first crystal structure of a Wnt protein bound to a Wnt receptor was made possible via the stability of a frog ligand-human receptor complex in solution, which will be further discussed in this microreview.

So, what is the big deal with Wnt? Clinically speaking, Wnt mutations, as well as mutations in molecules associated with Wnt signaling, are related to several hereditary diseases. A few examples include colon cancer, skin tumors, type II diabetes, obesity, osteoporosis (causes low bone mass), sclerosteosis (causes high bone mass), eye vascular defects, and tooth agenesis (Clevers and Nusse, 2012). How is Wnt associated with such an array of diseases? The general reason, as previously mentioned, is that cell proliferation, differentiation, and stem cell maintenance are regulated in the Wnt signaling pathway. Accordingly, Wnt research is active in areas such as cancer treatment, regenerative medicine, stem cell therapy, bone growth, and wound healing (Janda et al., 2012).

Before examining some recent research, it is helpful to have a basic understanding of the signaling pathway referred to as the canonical Wnt/β-catenin signaling pathway. There are two additional Wnt signaling pathways, but only the canonical pathway is relevant to this microreview. Perhaps of student interest, the canonical pathway is introduced in Chapter 20 of Essential Cell Biology (3rd edition) by Alberts et al. However, the textbook is not completely accurate, as recent research has introduced new findings that modify the pathway. Moreover, Wikipedia has not been updated. Please see Figure 1 for an overview of the Wnt/β-catenin signaling pathway.

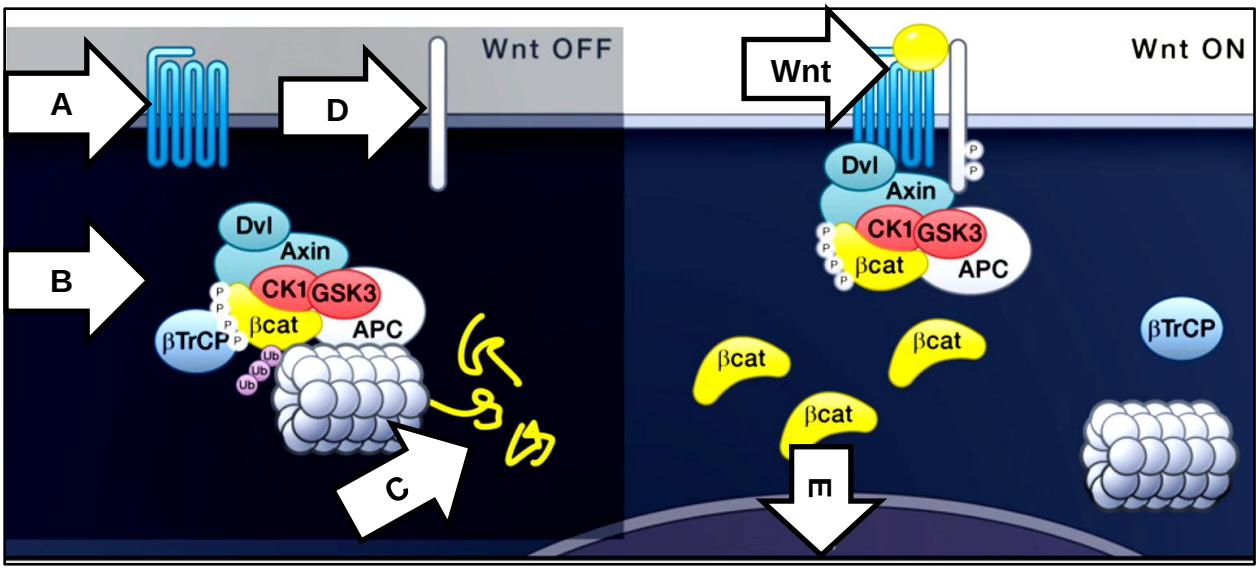

Figure 1. Schematic of the canonical Wnt/β-catenin signaling pathway inactive (left) and active (right). Arrow A is pointing to the Frizzled (Fz) receptor, which is a 7-transmembrane protein. Wnt binds to Fz outside of the cell (notated by the Wnt arrow) to activate the signaling cascade (right). In the absence of Wnt, the destruction complex that arrow B points to delivers β-catenin to the barrel shaped proteasome; arrow C points to degraded β-catenin. Note that the destruction complex resides in the cytoplasm. Arrow D points to LRP 5/6, a single-pass transmembrane protein, which associates with Wnt and the destruction complex when Wnt binds Fz. Once the pathway is activated, the destruction complex becomes saturated with β-catenin, which allows β-catenin accumulation. Note that βTrCP does not associate with the Wnt-ON complex, which prevents β-catenin from being ubiquitinated (marked for destruction). When β-catenin accumulates, it enters the nucleus (notated by arrow E) where it influences gene expression. *This schematic was derived from Clevers and Nusse 2012, and modified by the author of this microreview.*

Recent Progress

Janda et al. (2012) went down in history with their recent publication by submitting the first X-ray crystal structure of a Wnt protein bound to a Frizzled (Fz) receptor. They utilized a Wnt protein from a frog as the ligand for a human Fz receptor, which marked creation of the first stable Wnt-Fz complex in solution, which is necessary for crystallization. The structure was resolved to 3.25 Angstroms (3.25 X 10^{-4} µm). The authors noted that based on primary amino acid sequences Wnts are not clearly related to any known protein folds. Indeed, the resolved Wnt protein was asserted unique, as well as the manner of Wnt-Fz binding. The Wnt structure was likened to a hand with a distinct thumb and index finger projecting from the palm. The finger and thumb are both used to bind Fz. The thumb was shown to be dominated by a lipid. It was previously known that Wnt proteins are lipid modified in the endoplasmic reticulum by a gene called Porcupine. However, prior to this study the role of the lipid was only speculated. This study confirmed that the lipid portion of Wnt binds Fz. Moreover, without the lipid modification, Wnt remains in the cytoplasm, and does not activate the signaling pathway (Clevers and Nusse, 2012).

More Wnt advances came by way of research from Koo et al. (2012), who discovered that RNF43 and ZNRF3, homologous tumor suppressors, are ligases that mark Fz for destruction on intestinal stem cells. Specifically, RNF43 and ZNRF3 ubiquitinate Fz, which leads to internalization of the receptor, followed by endocytosis via lysosomes. When the researchers deleted RNF43 and ZNRF3 genes individually, they did not observe significant changes. However, simultaneous deletion caused adenomas (cancer precursors) to form. Koo et al. noted that RNF43 mutations have been observed in two colorectal cancer cell lines.

An earlier study showed a mechanism by which ZNRF3 is regulated in the Wnt pathway. A protein called R-spondin had been classified as a stem cell growth factor involved in Wnt signaling, but how R-spondin worked was not clear. Hao et al. (2012) demonstrated that R-spondin binds ZNRF3, which inhibits its ubiquitinating activity. When ZNRF3 is inhibited, the number of Fz receptors increase, thus enhancing Wnt signaling.

The final study examined in this microreview generated five findings that provide new understandings of how the canonical Wnt signaling pathway operates, hence a Wnt revolution (Li et al., 2012). The following is an outline of the findings, which will be further discussed in the next section:

1. Wnt signaling does not change the composition of the Axin complex.
2. Wnt signaling does not inhibit GSK3 or CK1.
3. β-TrCP acts within the intact Axin complex.
4. β-catenin is removed directly from the intact Axin complex upon degradation.
5. In APC mutant colorectal cancer, the Axin complex remains intact.

The molecules referred to in this outline are shown in Figure 1, which is the revised version of the canonical pathway, and incorporates the changes described above.

Discussion

Li and her team receive the "Most Productive Wnt Research Paper of the Year" award (my personal assessment). They pumped out five discoveries that contradict numerous ideas about Wnt signaling cascade functions, including what is currently taught in college textbooks. But this is a good thing, this is science! With a better understanding of how the canonical pathway functions, new targets can be honed for the treatment of myriad diseases related to mutations that cause over-/under-expression of components involved in this pathway. I have chosen to not include the obsolete diagrams of the canonical pathway because they are irrelevant—what is important is how it does work. This is not to say that knowing how it should not work is unimportant. After all, invoking point mutations and gene knock-outs have unequivocally led to advances.

Perhaps I am being biased toward Li and her team, of whom Hans Clevers was a notable member. Admittedly, before writing this microreview, I had never heard of a Wnt. As I dug into the literature, I quickly learned that the name Hans Clevers was tacked on to numerous recent publications. Moreover, many of his publications appear in prominent scientific literature, e.g. Science, Nature, and Cell. Clevers, who represents Hubrecht Institute in the Netherlands, was involved in more than half of the papers used for this microreview, and his work was cited by all but one of the remaining references. Clevers has written at least three Wnt related reviews, two of which I read, and one I used for this paper. Another notable name is Roel Nusse, who appears to be a pioneer in the Wnt research field, a field which is fairly young—the first Wnt protein was discovered in the early1980s. The purpose of this information is to direct the reader to insightful literature if interested. After all, is that not a Microreviews goal, i.e. to stimulate the interest of future scientists or doctors by flash-exposure to important and current scientific topics?

On a near-final note, and somewhat of a disclaimer, I do consider all of the examined literature to be important. The combination of the structural research paper (Janda et al., 2012) and Li et al. (2012), make Wnt signaling research an exciting venue full of open questions, which can now be more specific/directed questions to probe. Some questions that wait to be answered, as proposed by Clevers and Nusse (2012), verbatim, include:

- What is the evolutionary origin of Wnt signals?
- What is the nature of Wnt as a signal?
- Where does Wnt signaling take place in cells?
- How is the stabilized form of β-catenin ferried into the nucleus?
- How does Wnt signaling coordinate cell fate changes with changes in cell shape and polarity?
- Is there a universal "stemness" property conferred to cells by Wnts?
- How much of the genome is Wnt controlled across various cell types?
- Are cancer stem cell behaviors controlled by Wnt signaling?
- Can we identify bona fide and effective Wnt inhibitors?

I close this microreview with The Unusual Case of Porcupine (Lum and Clevers, 2012) to demonstrate (and perhaps fuel) the spirit of the Wnt revolution. This paper highlights a landmark event by which a Wnt signaling molecule is being targeted in humans for the first time. Lum and Clevers note that the induction of Porcupine targeting in clinical tests is a bold and unusual move in medicine. Recall that Porcupine modifies Wnt proteins to make them functional. Thus, inhibiting lipid modification of Wnt will render the signaling pathway inactive. Despite what is known about Porcupine, Lum and Clevers note that our knowledge of Porcupine may be incomplete in regards to other aspects of cellular function that it may affect. Additionally, they strongly emphasize the challenge of developing a Porcupine antagonist. Nevertheless, the promising success of eliminating cancerous growth as seen in lab research, such as research reported in this microreview, has spurred the novel decision to proceed.

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