Telomere stability—Wnt it or lose it

Kerstin Bauer, Si Tao & K. Lenhard Rudolph

Increasing data connect Wnt/ β -catenin signalling to telomere maintenance pathways. In this issue of *EMBO reports*, Gilson and colleagues provide the first evidence that this interconnection involves β -catenin-dependent transcription of the telomeric-repeat-binding factor 2 (TRF2) protein [1], which is required for telomere capping of chromosome ends.

Telomeres in ageing and cancer

Capping of chromosome ends is essential for the stability of linear chromosomes. It requires a minimum length of telomeric DNA, which can be synthesized and elongated by the enzyme telomerase. In addition, functional telomeres depend on the coordinated expression of a group of proteins that bind to telomeric DNA. These proteins are known as components of the shelterin complex and contribute to several processes including (i) the formation of higher-order secondary structures at telomeres, such as telomeric DNA loops (T-loops) and G-quadruplexes [2], (ii) the inhibition of DNA damage responses at chromosome ends [3], and (iii) the replication of telomeric DNA during S-phase [4].

Research over the past decades revealed that telomeres influence both cancer formation and ageing. Telomeres shorten in humans during ageing due to the limited expression of the enzyme telomerase. Telomere shortening leads to an accumulation of dysfunctional telomeres inducing DNA damage checkpoints such as p53-dependent senescence, or apoptosis [5] and p53-independent crisis. These checkpoints represent potent tumour-suppressor mechanisms that limit the capacity of transformed cells to expand clonally and to progress into macroscopic tumours. However, as a drawback, activation of the same checkpoints in response to age-dependent telomere shortening can limit the maintenance of functional stem cells and tissue

Fig 1 | Interaction between Wnt/ β -catenin signalling and telomere stabilizing pathways. Wnt/ β -catenin signalling could influence stem cell function, ageing and cancer by activating *TERT* and *TRF2* transcription. Activation of these two genes can lead to telomere stabilization, thus increasing the proliferative capacity of stem cells and cancer cells. In addition, *TERT* can activate the transcription of β -catenin and amplify β -catenin-dependent transcription of target genes. *TERT*, telomerase reverse transcriptase; *TRF2*, telomeric-repeat-binding factor 2.

homeostasis during ageing [5]. In addition, accumulation of telomere dysfunction also contributes to the development of cancer in ageing tissues by inducing chromosomal instability and by increasing the selection for abnormally growing cells with defective checkpoint responses [5].

Tissue homeostasis depends crucially on stem cell self-renewal, proliferation and differentiation to compensate for the loss of cells during ageing and in response to injury and diseases. In adult tissues, stem cell function is regulated by growth signalling pathways that also instruct the development of tissues and organs during embryogenesis. There is evidence that one of these signalling pathways, the Wnt/ β -catenin pathway interconnects with the catalytic subunit of the telomerase holoenzyme—telomerase reverse transcriptase (*TERT*). This represents the limiting

component for telomerase activity and telomere maintenance in somatic human cells. Beside its role in tissue maintenance and ageing, the interconnection between Wnt and telomerase could be relevant for cancer development as both Wnt/ β -catenin signalling and telomerase activity are upregulated in multiple human tumours.

TERT activates Wnt and vice versa

In recent years, it was discovered that *TERT* activates Wnt/ β -catenin transcription and stem cell proliferation independently from its 'reverse transcriptase' domain, which is required for elongation of telomeric DNA. Specifically, *TERT* overexpression resulted in the activation of hair follicle stem cells in transgenic mice, thereby mimicking the phenotype of other mouse models with induced β -catenin signalling [6]. Conversely, deletion of *TERT* resulted in

defects in the formation of the anterior–posterior axis during frog embryogenesis, resembling phenotypes induced by impairments in Wnt/ β -catenin signalling [7]. Importantly, TERT interacts directly with the β -catenin transcriptional complex by binding to BRG1 (also called SMARCA4; SWI/SNF-related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4)—a chromatin remodelling factor [7]. However, TERT not only activates Wnt/ β -catenin signalling, this signalling in turn activates the transcription of TERT—resulting in improved telomere maintenance in embryonic stem cells [8]. β -catenin activates transcription of TERT by interacting with KLF4, one of the four factors promoting reprogramming of somatic cells into induced pluripotent stem cells [8].

Together these results suggest that enhanced Wnt/ β -catenin signalling could influence stem cell function in two ways: (i) enhancing proliferative capacity by prolonging telomere maintenance through transcriptional activation of TERT, and (ii) modulating stem cell activity by activating a β -catenin/TERT-dependent feedforward loop that amplifies β -catenin-dependent gene transcription. Both regulatory loops could contribute to tumorigenesis. Along these lines, β -catenin was shown to bind directly to the TERT promoter in a murine *in vivo* model of intestinal tumour formation [8] and to contribute to the activation of TERT transcription and telomere maintenance in human cancer cell lines [9].

Wnt activates transcription of *TERF2*

TRF2 is an essential factor in the shelterin complex required for the formation of T-loops, telomere capping [2], replication of telomeric DNA during the cell cycle [4] and the suppression of ataxia telangiectasia mutated (ATM)-dependent DNA damage responses at telomeres [3].

In this issue, Gilson and colleagues provide several lines of experimental evidence that β -catenin activates transcription of TRF2: (i) both the human and murine *TERF2* genes contain TCF/LEF-binding sites in their regulatory regions, (ii) TCF/LEF-binding

...Wnt/ β -catenin signalling might enhance stem cell function and tumour growth by transcriptional activation of *TERF2* and *TERT*...

sites are required to increase *TERF2* promoter activity in response to β -catenin activation, (iii) chromatin immunoprecipitation on human cancer cell lines and mouse intestinal tissue revealed a direct interaction between β -catenin and the TCF/LEF binding sites in the regulatory region of the *TERF2* gene, and (iv) endogenous levels of TRF2 are affected by the activity of the Wnt/ β -catenin signalling pathway. Importantly, the authors show that β -catenin-dependent induction of *TERF2* increases TRF2 binding at telomeres and this is associated with improved chromosome-end protection in human cancer cell lines. On the contrary, knockdown of β -catenin aggravates telomere dysfunction accompanied by decreased cell viability and increased senescence. These data suggest that Wnt/ β -catenin-dependent TRF2 induction might promote progression of chromosomally unstable tumours by enhancing telomere capping. In line with this hypothesis, the study provides correlative evidence showing that activating β -catenin mutations are associated with increased TRF2 expression in primary human hepatocellular carcinoma.

Future perspectives

The study by Gilson and colleagues discloses a new link between Wnt/ β -catenin signalling and TRF2-dependent telomere capping. Together with work on the bidirectional interaction between Wnt/ β -catenin and TERT, these data support the idea that Wnt/ β -catenin signalling might enhance stem cell function and tumour growth by transcriptional activation of *TERF2* and *TERT* (Fig 1), resulting in improved telomere capping and amplification of β -catenin-dependent gene transcription through TERT. To delineate the *in vivo* role of these regulatory loops for stem cell maintenance, ageing and carcinogenesis remains

a major challenge for future research. In terms of stem cell function and ageing, activation of Wnt/ β -catenin signalling might not be beneficial. Although the pathway is required for maintenance of adult stem cells [10], increased Wnt activity can lead to aberrant differentiation and exhaustion of stem cells during ageing [11,12]. The generation of genetic mouse models that specifically abrogate the capacity of TERT to amplify β -catenin-dependent gene transcription, or the capacity of β -catenin to activate *TERT* or *TERF2* transcription, could help to address the physiological role of these regulatory loops during ageing and tumorigenesis. Such studies might, in the end, provide a rational basis for the development of therapeutic approaches aiming to target interactions between β -catenin and telomere maintenance pathways, to either enhance stem cell function and organ homeostasis during ageing or impair the progression of tumours that depend on the activation of TERT and β -catenin.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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