How Wnt/β-Catenin, TGF-β/BMP signalling effect senescence via polycomb.

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Abstract

Aging and age related diseases are accountable for most of the deaths world-wide. Many of these age related diseases are associated with an increase in senescent cells. A combination of increased induction of senescence and lowered clearance of senescent cells by the aged immune system is accountable for this increase in senescence. The secretory phenotype of senescent cells affects the stem cell niche and lowers the stem cells ability to proliferate and differentiate, resulting in Impaired tissue regeneration. Polycomb related complexes have been shown to regulate senescence. Polycomb repressive complex 1 expression is associated with a reduction of senescent cell markers. In this paper polycomb related complexes and senescence are linked via Wnt/β-Catenin, TGF-β and BMP signalling. The increased amounts of senescent cells in aged tissue effects TGF-β, BMP and Wnt signalling. These alterations in TGF-β, BMP and Wnt signalling lead to different polycomb repressive complex (PRC) compositions. Different PRC compositions again affect TGF-β, BMP and Wnt signalling. In this review it is shown that the increased amounts of senescent cells alter the balance in TGF-β, BMP and Wnt signalling in a way, which stimulates PRCs to facilitate senescence and thereby create an unwanted positive feedback loop.

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1.0 Introduction

100.00 people world-wide daily die due to aging and age related diseases (De Grey, A. D. 2007). Age related diseases include a wide variety of diseases: different cancers, neurodegenerative diseases (e.g. Alzheimer), cardiovascular diseases, diabetes type 2 and more. (Smith, B. D et al, 2009; Mrak, R. E. et al, 1997; Ostan, R. et al, 2016). Senescent cells are thought to negatively contribute to pathology of many of these age related diseases, however not every. Senescent cells prevent the development of cancers and cancers are a major contributor to the amount of age related deaths (Hashimoto, M. et al, 2016). Nevertheless, senescence has been associated with many of the age related diseases such as, COPD, cardiovascular disease, diabetes type 2, neurodegenerative diseases and more (Baker, N. et al, 2015; Kato, R. et al, 2016; Crowe, E. P. et al, 2016). Senescent cells can contribute to the disease pathology by the secretion of several Factors. These factors that senescent cells secrete affect tightly regulated processes, which include differentiation, migration, cell growth, blood vessel formation and tissue architecture. Senescent cells also secrete pro-inflammatory cytokines which contribute to the chronic inflammation observed in some of the age related diseases (Turinetto, V. et al, 2016). Since senescence plays a role in many age related diseases (Baker, N. et al, 2015; Kato, R. et al, 2016; Crowe, E. P. et al, 2016), it is important to understand the underlying mechanisms which drive cells into senescence. Understanding these mechanisms driving senescence, can give a more complete picture on how age related diseases can develop and might be useable to delay the onset of age related disease.

Sustained and irreversible tissue damage triggers cellular senescence via the induction of tumor-suppressor pathways $p19^{arf}$ (P14 in humans)/p53 and $p16^{ink4a}$. Activation of $p19^{arf}$/ p53 and $p16^{ink4a}$ induces and maintains a cell-cycle arrest, which prevents the development of cancers (Hashimoto, M. et al, 2016). Senescence therefore is not an unwanted process. However, aged tissue is characterised by an accumulation of senescent cells. This accumulation of senescent cells and the sub sequentially increase of factors secreted by the senescent cells limit tissue regeneration by preventing stem-cell proliferation and differentiation to restore cell-loss (Cheung, T. H. et al, 2013). There are multiple factors that contribute to this accumulation of senescent cells. On factor is the aged immune system that is less effective in clearing senescent cells (Cheung, T. H. et al, 2013). Besides less clearance of senescent cells, there is also an increased induction of senescent cells in aged tissue. An increase in age related oxidative stress and dysregulations of key differentiation regulatory factors (e.g. Runx2, C/EBPα, and PPARγ) contribute to the accumulation of senescent cells by inducing senescence (Turinetto, V. et al, 2016). Since polycomb is a key regulator of cell differentiation, a role of polycomb

related complexes in regulating/influencing senescence seems very likely. It is therefore not surprising that several connections between polycomb and senescence have been made.

Polycomb related complexes play a role in cellular senescence. Polycomb repressive complex 1 (PRC1) is a known senescence regulator that acts on the *INK4A/ARF* locus, where it supresses P16 ^{Ink4a}, p19 ^{Arf} and p53 activity. By limiting the P16 ^{Ink4a}, p19 ^{Arf} and p53 expression, PRC1 is able to reduce the amount of senescence (Moon, J. et al, 2011). Additionally, the composition of secreted proteins by senescent cells can be altered due to PRC activity. For instance, PRC1 related BMI1 activity limits the amounts of secreted monocyte chemoattractant protein-1 (MCP-1). MCP-1 secretion is increased in senescent cells (Jin, H. J. et al, 2016).

However, a less explored possibility is that Polycomb and senescence are also connected through developmental pathways, which is the focus of this essay. Developmental pathways include Notch, Wnt/β-Catenin, TGF-β, BMP and Shh/Patched signalling, which are involved in controlling tissue differentiation (Hsu, Y. C. et al, 2012). Polycomb is known to affect gene expressions of key developmental pathways (O'Hagan, H. M. 2014). On the other hand, there are several studies showing developmental pathways are being affected by senescent cells. Senescent cells can secrete factors that affect developmental pathways and thereby affect the niche of surrounding cells. (Li, Z. et al, 2016; Kim, H. et al, 2015; Gargiulo, G. et al, 2013).

The main question that will be addressed is: Are polycomb related complexes able to affect senescence by modulating TGF-β, BMP and/or Wnt signalling? And likewise, the effects of TGF-β, BMP and Wnt signalling on polycomb will be discussed to get a complete picture about the implications of altered TGF-β, BMP and/or Wnt signalling on polycomb. This paper will start with an introduction on senescence, followed by the effects of TGF-β, BMP and/or Wnt signalling on senescence. Part two starts with an introduction on the polycomb group followed by the effects of TGF-β, BMP and/or Wnt on polycomb related complexes. Lastly, the found results will be combined to conclude how senescence is effected by polycomb associated alterations in TGF-β, BMP and/or Wnt signalling.

2.0 Senescence and ageing.

Stem cells are undifferentiated, long lived cells that are able to produce daughter cells and self-renew. Most tissue contains resident stem-cells, which proliferate to compensate for tissue loss (Cheung, T. H., & Rando, T. A. 2013). A small proportion of the stem-cells remains in a quiescent state, a state where the stem cell is kept in a reversible G0 and thus does not proliferate or differentiate. However, loss or deregulations of quiescence results in imbalances in progenitor cell populations, which limits

effective tissue regeneration and repair (Cheung, T. H., & Rando, T. A. 2013). The stem-cell maintains the quiescent state via intrinsic mechanisms and the stem-cell niche. When tissue is damaged this results in changes in the stem-cell niche. These changes in the stem-cell niche allows the quiescent stem cells to re-enter the G1 phase. When entering the G1 phase the fate of the stem cell is determined. It can either differentiate, proliferate and re-enter the quiescent state or become a senescent cell (figure 1) (Cheung, T. H., & Rando, T. A. 2013). Aging is characterised by an accumulation of senescent cells. Removal of senescent cells has been shown to delay age related phenotypes in mice and increased the mice's life-spawn. (Baker, D. J. et al, 2011).

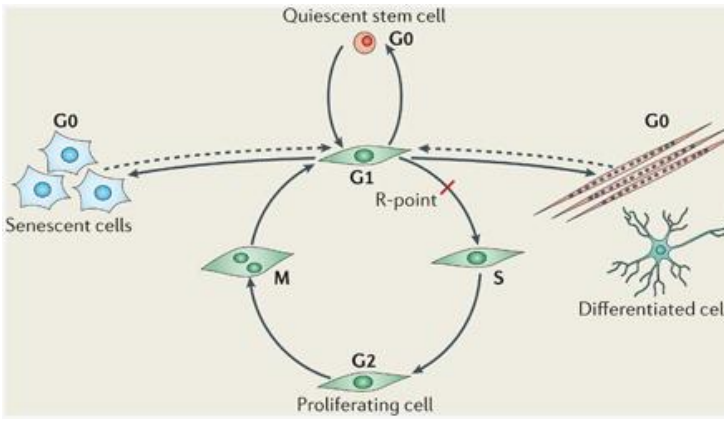

Figure 1. A schematic overview of quiescent stem cells, changes in stem cell niche enables the stem cell to enter the G1 phase. In the G1 phase the fate of the stem cell is determined. It can differentiate, proliferate and return to the quiescent state or become a senescent cell (Cheung, T. H. et al, 2013).

Senescent cells secrete factors that affect the stem cell niche and limit the stem cells to respond effectively against tissue loss. The upregulation of various protein expression by senescent cells is termed as the senescence-associated secretory phenotypes (SASPs). A study in which gene and protein expression of secretory proteins was compared between presenescent and senescent cells found several proteins to be significantly more secreted by senescent cells (Coppé, J. et al, 2008). The proteins that are increased secreted by senescent cells include; inflammatory and immune-modulatory cytokines and chemokines (e.g., IL-6, −7, and −8, MCP-2, and MIP-3a), growth factors (e.g., GRO, HGF, and IGFBPs). It was also shown that shed cell surface molecules (e.g., ICAMs, uPAR, and TNF receptors) and p16, p53 and p21 are highly expressed in senescent cells (Coppé, J. et al, 2008). SASPs influence the stem cell niche, so can IL-6 secreted by senescent cells disrupt the quiescent state of stem cells (O'Hagan-Wong, K. et al, 2016). The SASPs can also signal in an autocrine manner, where several components of the SASPs have been shown to help in maintaining a senescent state (IL-6, IL-8, and GROα) (van Deursen, J. M. 2014).

2.1 Senescence and BMP/TGF-β signalling

2.1.0 The BMP Pathway

BMPs are members of the TGF-β superfamily and signal via BMPRI and BMPRII. The presence of a BMPR ligand induces the BMPR1 and BMPRII to form a ternary holocomplex. This complex can induce a response dependently or independently of Smads. In the smad independent pathway several MAPK kinases are recruited (e.g. ERK, p38 and JNK) (de Vinuesa et al, 2016). The smad dependent pathway goes via the phosphorylation of SMAD1, 5, 8. The p-Smads 1, 5 and 8 form a hetero-oligomeric complex with Smad4 (Rahman, M. S. et al, 2015). The smad hetero-oligomeric complex is able to translocate to the nucleus where it induces the gene transcriptions of various genes involved in proliferation and differentiation, such as JAG-1, ENG, BMPRII, ALK3 and 6, SMAD 6/7, VEGFR1 and downregulation of VEGFR2, VEGF, FGRF (figure 2) (Daly A. C. et al, 2008).

2.1.1 The TGF-β pathway

Three isoforms of TGF-β have been identified in mammals TGFβ (β1, β2, and β3). All isoforms play a role in wound healing, inflammation and development. TGFβ1 plays a dominant role during wound repair, while TGFβ2 and β3 play a more dominant role during development and scarless wound healing (Tandon, A. et al, 2010). TGFβ binds to the TGFβ receptor type 2 (TGFβR2), which leads to the activation of the TGFβ receptor type 1 (TGFβR1), in as similar way as BMP activates the BMPRs. Activation of the TGFβRs results also in either a Smad dependent or Smad independent signalling cascade. In the smad independent pathway MAPK kinases (e.g. ERK, p38, and JNK) are recruited and in some specific cell types this signalling can include PP2Aphosphatase and RhoA activity (Tandon, A. et al, 2010). The Smad dependent pathway includes the phosphorylation of Smad 2 and Smad 3. P-smad 2/3 requires Smad 4 to translocate to the nucleus. Since Smad 4 is required for both TGF-β related p-Smad 2/3 and BMP related P-Smad 1, 5 8 to translocate to the nucleus, there is competition over the smad 4. A study showed that the inhibitory effects of BMP on TGF-β were elevated due to excessive amounts of SMAD4 (Candia, A. F. et al, 1997). That TGF-β and BMP antagonise each other was further shown in human lung fibroblasts, where BMP4 inhibits TGF-β1 signalling (Pegorier, S 2010). It was later shown that TGF-β1 inhibits BMP signalling in human pulmonary artery smooth muscle cells (Upton, P. D. & Morrell, N. W. 2013).

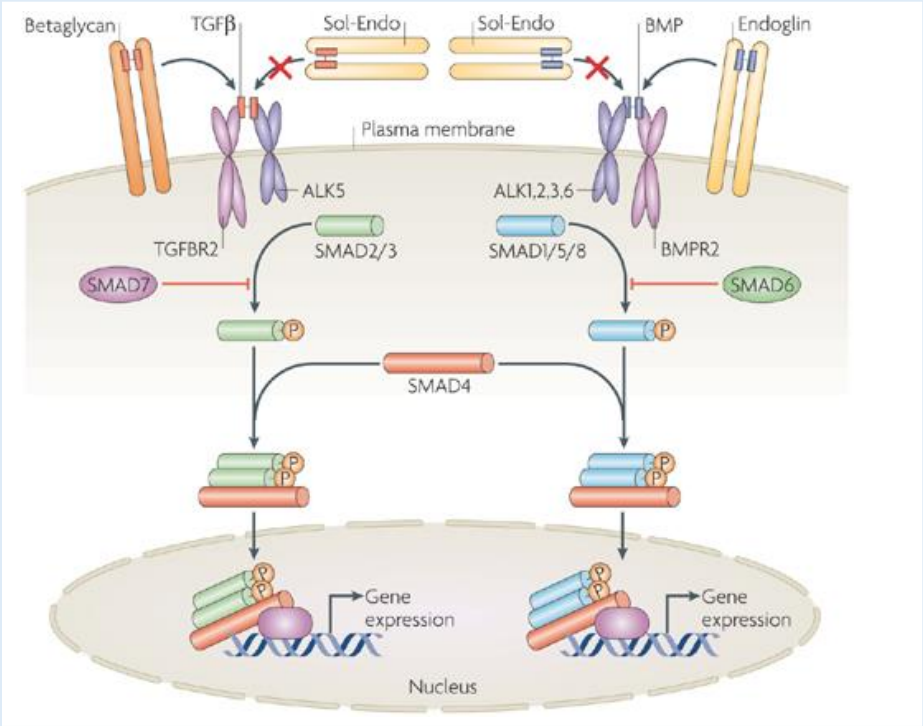

Figure 2. BMPRI / BMPRII activation leads to the phosphorylation of Smad1, 5 8. P-smad1, 5, 8 forms a complex with smad 4 which is able to translocate to the nucleus, where it induces the transcription of several genes (ten Dijke, P. & Arthur, H. M. 2007).

2.1.3. The role of TGF-β and BMP in senescence.

Elevated levels of TGF-β1 were found in the human corneal epithelium (HCE) of elderly donors, compared to young donors. In line with the age related increase of senescence, senescent cell marker p16 was also found to be higher expressed in the HCE of elderly donors (Li, Z. et al, 2016). Treating HCE cells with TGF-β1 increased the expression of SA-β-gal, a senescent cell marker. This indicates that TGF-β1 can induce senescence (Li, Z. et al, 2016). Also in Bone marrow derived mesenchymal stem cells TGF-β1 was shown to induce SA-Gal activity in a dose dependent way (Wu, J. et al, 2014).

Despite the inhibitory effects of BMP on TGF-β signalling and therefore expected opposite effect of BMP on senescence, it was shown that BMP4 treatment induces SA-Gal activity in A549 cancer cells. Smad 1 expression was upregulated due to BMP4 treatment, however only overexpressing smad 1 did not lead to senescence but to cell death (Buckley, S. et al, 2004). BMP2 has been shown to upregulate the gene and protein expression of senescent cell marker p21 in human breast cancer cells (Pouliot, F, & Labrie, C. 2002). Also in this study the researchers postulated that phosphorylation of smad1 can induce senescence. When only Smad1 was expressed, this led to an induction of p21. In combination with Smad4 and BMP2, this induction was higher (Pouliot, F, & Labrie, C. 2002). In a study where the

SASPs was investigated in senescent NRK-52E cells, it was found that inhibition of MAPK activity attenuated the induction of secreted alkaline phosphatase (SEAP). SEAP expression is closely correlated to p16 and p21 expression and therefor is also senescence marker (Gu, L. & Kitamura, M. 2012). Since MAPK activity can be induced by TGF-β and BMP via the smad independent pathways (de Vinuesa et al, 2016; Tandon, A. et al, 2010), this implies also a smad-independent induction of senescence.

2.2. Senescence and the Wnt pathway

2.2.0. The Wnt pathway.

The Wnt family is involved in cell growth, differentiation, function and death and consists of 19 highly conserved genes in humans. The term “Wnt” is a combination of wingless and int. Int was first found and described in a mouse mammary tumor. Later, wingless was sequenced in the Drosophila and it was discovered that int-1 and wingless are homologues, which led to the name Wnt (Wang, Y. et al, 2014). The Wnt receptor can be activated by a variety of agonists, including: Noggin, Frizzled-4, the R-spondin family and several Wnt types. The activation can lead to the induction of three distinguishable signalling pathways. Firstly the Wnt canonical pathway or one of the two noncanonical pathways; the Ca2+ pathway and PCP pathway (Wang, Y. et al, 2014). The canonical pathway is the best described Wnt pathway, in this pathway activation of the WNT receptor prevents the phosphorylation of β-catenin by the Gsk3β complex. Inhibiting the phosphorylation of β-catenin impairs the degradation of β-catenin, which thereby enhances β-catenin’s ability to translocate to the nucleus (figure 3) (Bafico, A. 2001). In the nucleus β-catenin interacts with LEFs and Tcfs to induce gene expressions of several target genes, including c-Myc, Cyclin D1, Surviving and HDAC (Subramaniyan, B. et al, 2016)

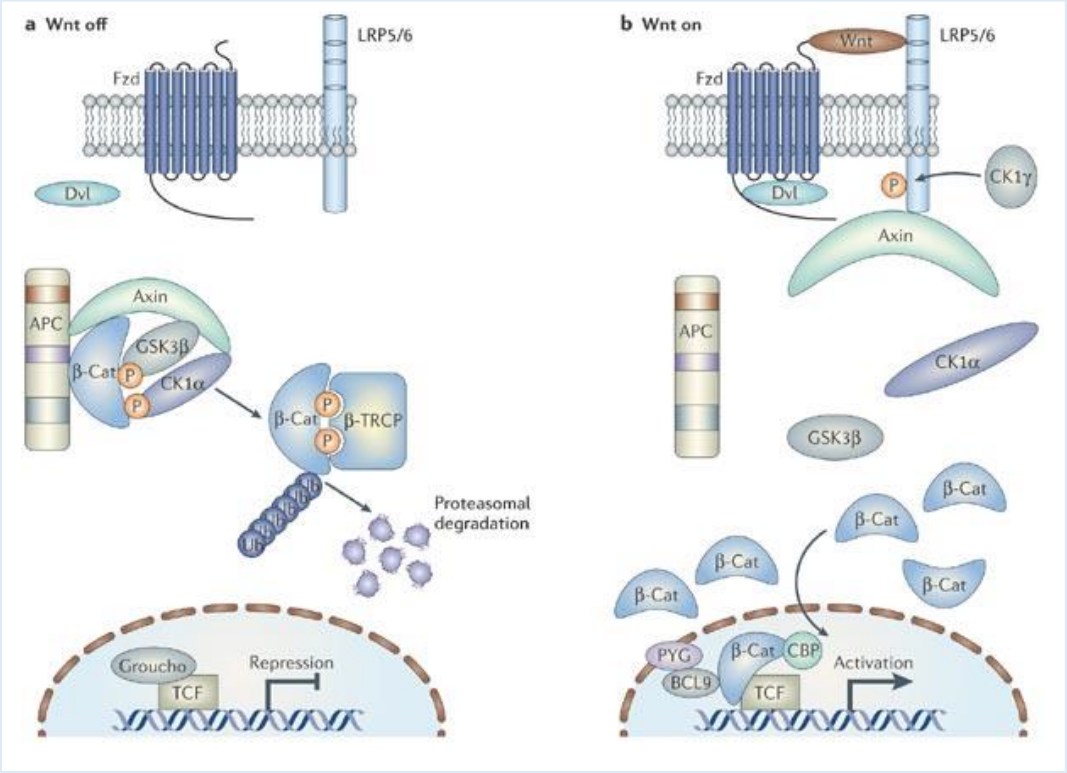

Figure 3, on the left side the inactive Wnt pathway. The absence of a Wnt ligand leads to the phosphorylation and degradation of β-catenin facilitated by the Gsk3β complex. On the right side the activated Wnt pathway, were phosphorylation of β-catenin is inhibited. Due to this, β-catenin is not degraded and can translocate to the nucleus (Barker, N. & Clevers, H. 2006).

2.2.1. The Wnt pathway in senescence

A study performed to investigate Wnt signalling in senescent cells, found that Wnt2a mRNA was downregulated in senescent human fibroblasts. Consequently, GSK3β kinase activity was significantly upregulated in senescent human fibroblasts. In line with the increased GSK3β kinase activity, less soluble β-catenin was observed. This study shows the canonical Wnt pathway is effected in senescent human fibroblasts (Ye, X. et al, 2007). Later in this study, Wnt2 was knock-out in primary human WI38 fibroblasts by the use of shRNAs. It was found that Wnt2 inhibition induces senescence in these cells. It was stated that this is evidence that Wnt inhibition is not a consequence of senescence, but that impaired Wnt signalling rather induces senescence (Ye, X. et al, 2007). They strengthened this hypothesis by overexpressing Wnt3a (it was not managed to overexpress Wnt2a), where they observed that Wnt3a overexpression delayed the onset of senescence (Ye, X. et al, 2007).

Subsequent studies also found an effect of senescent cells on Wnt signalling. A quantitative proteomic analysis of the proteins secreted by senescent human fibroblasts, showed an increase of secreted Frizzled-related protein 1 (SFRP1). SFRP1 can bind to Wnt, and thereby inhibit Wnt signalling

(Drescher, U. 2005; Elzi, D. J. et al, 2012). Reducing SFPR1 expression via SFRP1 RNA interference (RNAi) or a SFRP1 neutralizing antibody prevented the induction of senescence (Elzi, D. J. et al, 2012). SFRP1 can bind to Wnt, and thereby inhibit Wnt signalling (Drescher, U. 2005). This indicates that senescent cells can maintain their senescent state and induce senescence in other cells via the secretion of SFRP1.

However, there are studies published that show a complete opposite. In a study Mesenchymal stem cells (MSCs) were treated with old or young rat serum (ORS, YRS). ORS was found to significantly upregulate the amount of SA-β-gal–positive senescent cells compared to YRS treated MSCs. It was also shown that ORS induces expression of the canonical Wnt pathway. When they treated YRS treated MSCs with Wnt3a, it was shown that Wnt3a induced SA-β-gal expression. DKK1 (a Wnt inhibitor) and si-β-catenin could prevent the Wnt3a induced increase of SA-β-gal expression (Zhang, D. Y. et al, 2011).

High Wnt-1 expression has also been shown to induce senescence in hair follicle stem cells (HFCs) (Castilho, R. M. et al, 2009). In this study conducted by Castilho et al, it was noted that the Wnt-1 expression pushes quiescent HFCS out of their quiescent state, which resulted in differentiation and ultimately HFC stem cell depletion. No stem cells were observed after prolonged Wnt-1 treatment (Castilho, R. M. et al, 2009). It seems plausible that pushing quiescent SCs out of the quiescent state automatically results in an increased change of observing senescent cells. This can be a possible explanation for the conflicting results observed due to Wnt treatment on senescence. Furthermore, only 100ng/ml Wnt3a was capable of inducing SA-β-gal expression, lower concentrations of Wnt3a had no effect (Zhang, D. Y. et al, 2011). These high concentrations of Wnt3a makes it likely that all quiescent cells leave their quiescent state and therefore have a change in becoming a senescent cell.

Despite the conflicting results, it can be stated that low Wnt signalling induces senescence and that Wnt signalling is negatively affected by senescent cells via the secretion of SFPR1 (Ye, X. et al, 2007). Prolonged and high expression of Wnt1 and Wnt3a have also been shown to also induce senescence (Zhang, D. Y. et al, 2011; Castilho, R. M. et al, 2009). In conclusion, properly regulated Wnt signalling is important in preventing the accumulation of senescent cells.

3.0 The function of polycomb genes

During development of the Drosophila embryo several distinct segments are being formed. The identity of these segments are specified and maintained by homeotic (Hox) genes. Mutations in Hox genes can result in unusual phenotypes, as the case in the Hox mutant Antennapedia (Antp). Antp mutants developed legs instead of antennae on their heads (Lanzuolo, C & Orlando, V. 2012; Lewis, E. B. 1978). Polycomb genes have been found to play an important role in regulating the expression of

Hox genes. It was shown that polycomb group (PcG) mutated Drosophila display defects in body-patterning. Improper Hox gene expressions observed in these mutants lead to the conclusion that PcG derived proteins play a role in the regulation of Hox gene expression (Franke, A. et al. 1992).

Nowadays much research on the polycomb group has been performed. It was observed that PcGs are highly evolutionary conserved in animals and in plants. Homologues of PcGs have even been found in unicellular organisms (Huang, Y. et al. 2016). In humans two distinguishable PcG complexes have been well described, polycomb repressive complex 1 and 2 (PRC1 and PRC2) (Visser, H. P. et al. 2001). In humans the PcG plays a proficient role during development via the regulation of several key genes involved in developmental pathways. Besides development, PRC1 and PRC2 have also been linked to DNA repair, cell fate decisions, senescence and apoptosis (O'Hagan, H. M. 2014; Klauke, K. & de Haan, G. 2011).

3.1 How polycomb complexes silence genes.

In eukaryotic cells, histones are build out of two copies of histone proteins H2A, H2B, H3, and H4, forming an octamer. Histones play an important role in organising the DNA. DNA is a negatively charged polyelectrolyte and thus hinders the electrostatic DNA-DNA repulsion the compaction of the DNA. Histones are positively charged and thereby partly compensate for the electrostatic DNA-DNA repulsion, allowing DNA to be wrapped around histones and form nucleosomes (Korolev, N. et al, 2007). How tightly the DNA is wrapped around the histone, determines how well available the chromatin is for proteins to bind and induce gene transcription (Eberharter, A. & Becker, P. B. 2002). Each histone core unit contains a flexible N-terminal domain, named the histone tail. The histone tail make contact with wrapped DNA or other nucleosome core units. The histone tails are necessary in maintaining the wrapped state of the DNA, via positively charged lysine and arginine residues that stabilises the nucleosome structure (Korolev, N. et al, 2014). Post-translational modifications on histone tails effects the wrapping tightness of DNA wrapped around the histone and thereby the chromatin accessibility. The Post-translational modifications include phosphorylation, methylation and acetylation. Acetylation of lysine or arginine residues in histone tails make the chromatin more available, due to the loss of a positive charge, which makes the DNA wrapping less tight. Phosphorylations on the histone tails make the histone tail more negatively charged. According to the "histone code hypothesis", the addition of the negatively charged phosphate cause the chromatin fibres to decondensate, making the DNA more available for proteins to bind (Strahl, B. D. & Allis, C. D. 2000). Histone tail methylations make the chromatin less available for gene transcription (Eberharter, A. & Becker, P. B. 2002). Polycomb complexes methylate histone tails to limit chromatin accessibility

and thereby inhibit gene expression. Well-known targets of PRC2 and PRC1 are lysine 27 of histone 3 (H3K27) and lysine 119 of histone 2A *(Piunti, A., & Shilatifard, A. 2016)*

Firstly the PRC2 complex is recruited. PRC2 has been shown to mainly bind in CpG-rich domains and It is thought that transcriptionally silent unmethylated CpG islands are responsible for PRC2 recruitment (Riising, E. M. et al, 2014; Piunti, A., & Shilatifard, A. 2016). After recruitment, PRC2s controls the methylation mark on histone H3K27. Methylation of the lysine 27 in histone 3 disables CBP/p300 to acetylate the region and induce activity, thus PRC2 causes gene repression (Piunti, A., & Shilatifard, A. 2016). The methylations implemented by PRC2 can result in three different histone H3K27 methylation types, H3K27me1, 2 and 3. Each of the three H3K27 types are exclusively found in different regions. H3K27me1 is mainly found in gene bodies associated with actively transcribed genes, H3K27me2 in intergenic regions and H3K27me3 mainly at promotor sites of bivalent genes (Piunti, A., & Shilatifard, A. 2016).

PRC1 can be divided in two main subtypes, canonical and noncanonical. Canonical PRC1s contain a CBX subunit that can recognise the PRC2s catalysed H3K27me3. Upon recognition, PRC1s are responsible for H2AK119ub deposition which results in chromatin compaction and transcriptional silencing (figure 4) (Piunti, A., & Shilatifard, A. 2016).

The noncanonical PRC1s contain a RYPB instead of a CBX and therefore are PRC2 independent. A precise mechanism by which non-canonical is recruited and facilitates H2AK119ub is not known. It has been shown that noncanonical PRC1 is responsible for H2AK119UB independent from PRC2 and H3K27me3 (figure 3) (Tavares, L. et al, 2012; Piunti, A., & Shilatifard, A. 2016).

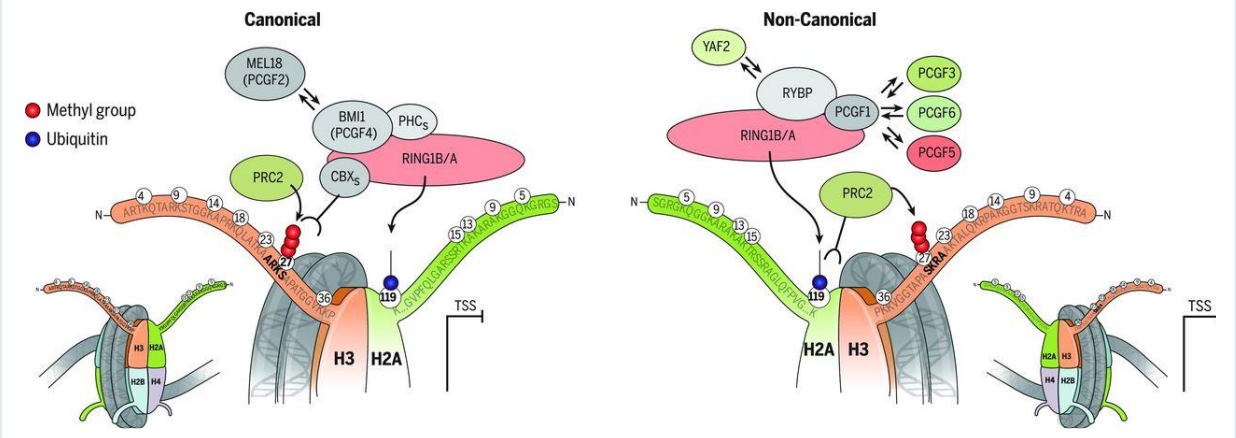

Figure 4: on the right canonical PRC1. CBX recognises the PRC2 inserted H3K27me3 resulting in H2AK119ub. On the left the non-canonical pathway, PRC1 containing RYBP instead of CBX which is able to implement H2AK119ub independently of PRC2 and H3K27me3 (Piunti, A., & Shilatifard, A. 2016).

Thus, PRC2 can inhibit gene transcription via the catalysation of H3K27 methylation and PRC1 mediates ubiquitin binding on H2AK119, either canonical or non-canonical. Both H2K27 methylation and H2AK119ub results in gene silencing *(Piunti, A., & Shilatifard, A. 2016)*.

3.2 Structure of human polycomb repressive complex 1 and 2.

PRC1s are build out of 5 core proteins from different protein families, however addition proteins can bind. The core protein families include CBX (Cbx2/4/6/7/8), PCGF (Bmi1, Mel18, and PCGF1/6), PHC (Phc1/2/3), RYBP/YAF2 and RING1/2 (Ring1A/B) (Bian, F. et al. 2016). Proteins of these families can be combined in varies ways to form a PRC1 (figure 5). Due to the variation that is possible, the PRCs1 are highly heterogeneous. Different compositions of the PRC1s leads to functionally different PRC1s, showing differences in target specificity and gene regulatory functions (van den Boom, V. 2013). There are PRC1 compositions that are not observed. When RYBP or YAF2 takes part in the complex, then this excludes CBX (Gil, J. & O'Loghlen, A. 2014). This leads to the distinguishability of two PRC1s, canonical and non-canonical PRC1. Canonical PRC1 contains CBX and PHC (4 total core proteins), while non-canonical PRC1s contains a RYBP or YAF2 (3 total core proteins) (Chen, T. & Dent, S. Y. 2014).

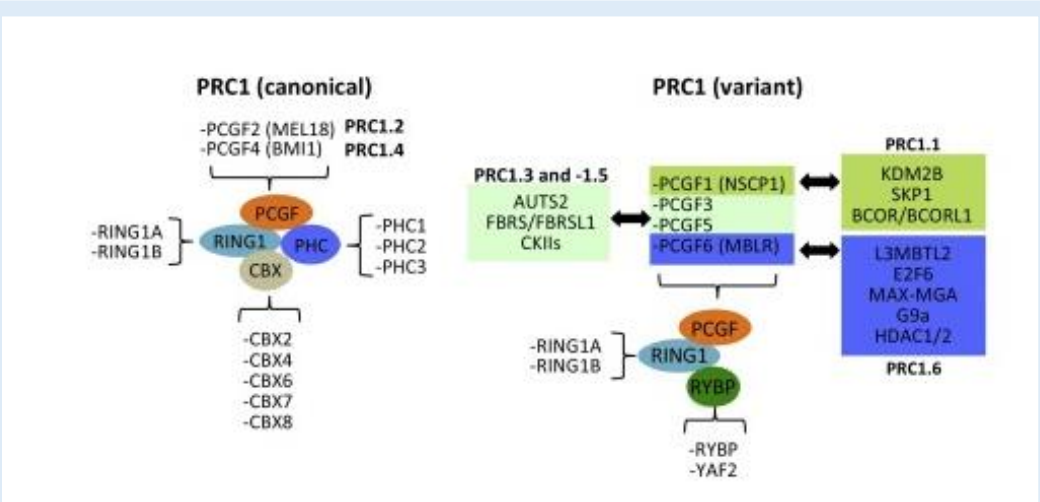

Figure 5: An overview of the diversity of PRC1s composition. Variation in PCGF, RING1, PHC and CBX result in functionally different PRC1 subtypes (Chen, T. & Dent, S. Y. 2014).

PRC2s can consist of seven proteins, out of which Ezh1/2, Suz12, Eed and RbAp46/48 are the core proteins that together are sufficient for enzymatic activity. Three additional proteins, AEBP2, Pcls and Jarid2 can take part in the PRC2 (figure 6). Each of the three additional proteins can affect the enzymatic activity of PRC2 in a different way. AEBP2 enhances enzymatic activity and gives more flexibility to PRC2s binding (Margueron, R. & Reinberg, D. 2011). Pcl1/2/3 are thought to be involved in the recruitment of PRC2s and affect PRC2s enzymatic activity. A third proposed function of Pcls is giving tissue specificity, since Pcls are expressed in a tissue specific manner. Jarid2's role in PCR2 function is less clear. Jarid2 inactivation leads to an impairment of PRC2 recruitment, surprisingly this does not lead to a significant reduction of H3K27me3 levels. (Landeira, D. et al, 2010; Margueron, R. & Reinberg, D. 2011). The chromatin remodelers DNMTs, HDAC1 and Sirt1 have also been shown to interact with PRC2, however these interactions have not been described in detail yet and the function of these interactions are unknown (Margueron, R. & Reinberg, D. 2011).

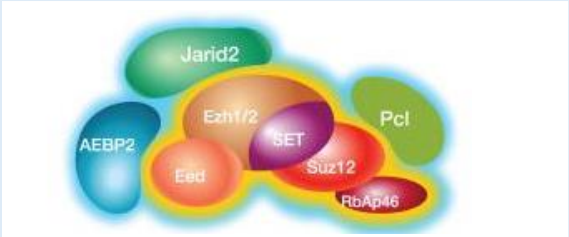

Figure 6: The PRC2 complex. A core composed of Ezh1/2, Suz12, Eed and RbAp46/48 combined with AEBP2, Pcls and Jarid2 (Margueron, R. & Reinberg, D. 2011)

The functions of different PRCs composition.

It was found that variations of CBX types within the PRC1 are associated with different processes. CBX7 is associated with self-renewal, inhibition of differentiation and downregulation of the CBX2, 4 and 8 expressions. While CBX2, 4 and 8 are mostly observed in differentiated cells. CBX 2, 4 8 downregulate the expression of CBX7. Other components of the PRC1 are associated with CBX type. CBX2 and 8 are more likely to be found in a complex together with BMI1 and PHC2 (O'Loghlen, A. et al, 2012). During differentiation complexes with a CBX7 are replaced by a PRC1 complex containing CBX 2, 4 or 8, thereby the PRC1 complex facilitates differentiation (Camahort, R, & Cowan, C. A. 2012; O'Loghlen, A. et al, 2012).

Not only during development is it essential that the desired PRC is functional. In a zebrafish study it was shown that upon amputating a part of the caudal fin, strong upregulation of BMI1, PCGF5B and

PCGF6 are observed in regenerating pars of the caudal fin. The expression of BMI1, PCGF5B and PCGF6 do not overlap and is specific for different parts of the caudal fin (figure 7) (Dupret, B. et al. 2016).

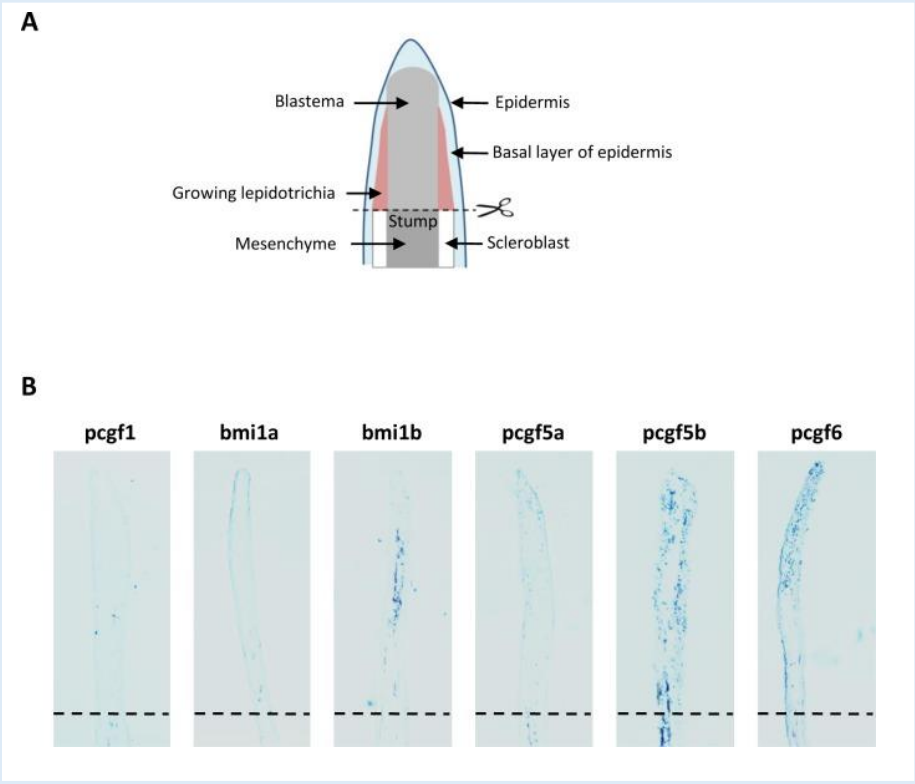

Figure 7: The regenerating zebrafish's caudal fin. Expressions of BMI1, PCGF5B and PCGF6 are highly expressed in different parts of the fin (Dupret, B. et al. 2016)

In this same study PCGF1$^{-/-}$ zebrafish were created by injecting the zebrafish embryo with TALEN mRNA. 35% of the zebrafish knocked-out for PCGF1 displayed signs of premature aging. These signs included a spinal curvature, which is a common age related morphological symptom in zebrafish combined with a higher senescence-associated β-galactosidase (SA-β-Gal) activity (Dupret, B. et al. 2016).

This study highlighted, that during tissue regeneration the expression of PRCs are essential. Tissue regeneration is often limited in age related disease, which is partly contributed to senescent cells (Baker, N. et al, 2015; Kato, R. et al, 2016; Crowe, E. P. et al, 2016). This raises the question whether PRC composition is changed by senescent cells and the factors senescent cells secrete. Altering the PRC composition could be a mechanism by which senescent cells are able to limit tissue repair and contribute to age related disease pathologies.

3.3 PRC1/2 and TGF-β/BMP signalling.

PRC1 has been shown to play an important role in the regulation of BMP and TGF-β signalling (Gargiulo, G. et al, 2013). Comparative immuno-stainings of cerebella from BMI1$^{-/-}$ and non-transgenic mice showed that the absence of BMI1 resulted in an increase of BMP signalling (Zhang, X. et al, 2011). P-smad1, 5 and 8 were stained in P0, P7 and P15 cerebella. In BMI1$^{-/-}$ P7 a significant increase of P-smad1, 5 and 8 expressions was shown. For P15 BMI1$^{-/-}$ cerebella this effect was not significant, however there still was an high p-smad 1, 5 and 8 expression visible in progenitor cells (Zhang, X. et al, 2011). The upregulation of p-smad 1, 5 and 8 was contributed to the increased BMP4 expression found in BMI1$^{-/-}$ cerebella. (Zhang, X. et al, 2011). The regulation of BMI1 on BMP signalling was further confirmed by a study showing that BMI1 binds on genes involved in TGF-β and BMP signalling (Gargiulo, G. et al, 2013). Besides the PRC1 related BMI1, also EZH has been shown to influence BMP signalling. Genomic sequencing of 0308 cells treated with EZH2 siRNA showed that BMPR1B expression was increased in EZH2 siRNA treated cells, compared to untreated cells (Lee, J. et al, 2008).

A different study, which also made use of BMI1$^{-/-}$ mice and non-transgenic mice showed that TGF-β1, TGF-βRII, pSmad2/3 and Smad4 were all upregulated in BMI1$^{-/-}$ mice. This shows that BMI1 downregulates the smad dependent TGF-β signalling pathway (Jin, J. et al, 2014). Treatment of ovarian cancer cells with TGF-β reduced the amounts of observed H3K27me3. This decrease in H3K27me3 was explained by a decrease in EZH2 protein expression (PRC2 related) and an increase in H3K27 demethylases (Cardenas, H. et al, 2016). It was further stated that TGF-β decreases H3K27me3 in the ZEB2 promoter (a target of smad complexes). It is implied that the decreased H3K27me3 in the ZEB2 promoter enables Smads to bind more effectively and thus make the smad-dependent TGF-β pathway more effective (Cardenas, H. et al, 2016). Upregulation of EZH2, supported by Eed and Suz12 (all PRC2 related) silences TGF-β1 gene expression via the induction of H3K27me3 mediated chromatin compaction (Lewandowski, S. L. et al, 2015).

It can be concluded that both BMP signalling and TGF-β are silenced by BMI1 and EZH2 expression (Zhang, X. et al, 2011; Lee, J. et al, 2008; Cardenas, H. et al, 2016). BMP signalling has not been shown to effect BMI1 or EZH2, however TGF-β has been shown to reduce EZH2 protein expression (Cardenas, H. et al, 2016). Thus, there is extensive crosstalk between PcG and TGF-β/ BMP signalling.

3.4 PRC 1/2 and WNT signalling.

PRC1 and 2 have been associated with WNT signalling, where WNT targets can upregulate the expressions of several components that take part in PRC1 or PRC2 (Galmozzi, E. et al, 2006; Yu, T. et al, 2012; Chen, Y. et al, 2016). BMI1 (PRC1) and SUZ12 (PRC2) are known to be downstream targets of the WNT pathway and expression of SUZ12 and BMI1 is upregulated due to WNT expression (Galmozzi, E. et al, 2006; Yu, T. et al, 2012). Also EZH2 expression is induced by WNT signalling. TCF4, a transcription factor induced by Wnt/β-catenin signalling, can directly bind to the EZH2 promotor and induce EZH2 expression (Chen, Y. et al, 2016). On the other hand is polycomb also able to affect the WNT pathway. Loss of PRC1 induces a strong upregulation of ZICs and other transcription factors that negatively regulate the β-catenin activity (figure 5). Ring1/2 of the PRC1 can bind to the promoter of ZICs, what leads to ZICs inhibition (Chiacchiera, F. et al, 2016). The inhibition of ZICs leads to an increase in β-catenin activity, due to the loss of ZICs repression of the β-catenin activity (Chiacchiera, F. et al, 2016).

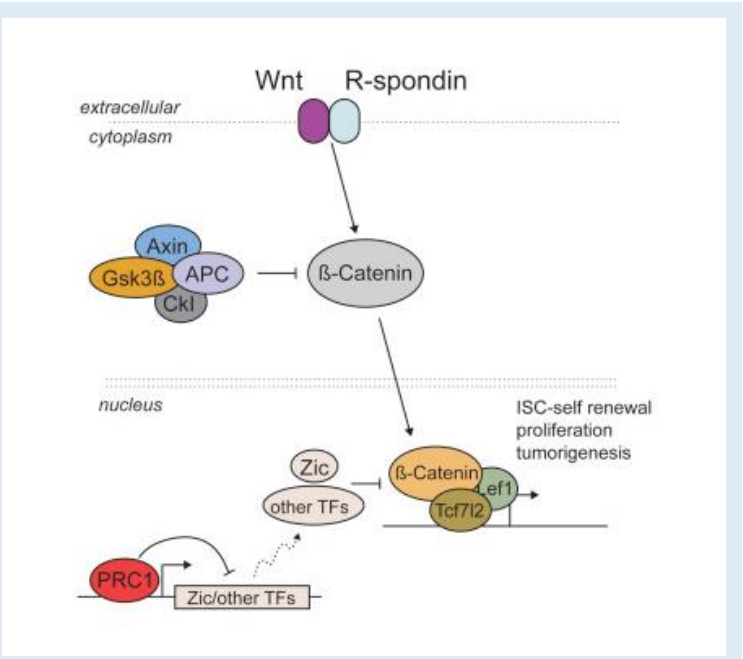

Figure 8: Loss of PRC1 leads to an increase in Zic 1 expression, which inhibits ß-catenin activity (Chiacchiera, F. et al, 2016).

EZH2 activity increases the expressions of c-Myc driven genes, a downstream target of WNT signalling (Shi, B. et al, 2007). Since EZH2 is a WNT target as well (Chen, Y. et al, 2016), this implies a positive feedback loop.

CBX7 induces expression of the distal promotor of Dickkopf-1 (DKK-1), resulting in an increased expression of DKK-1 (Kim, H. et al, 2015) DKK-1 is a WNT-antagonist (Bafico, A. et al, 2001). Thus induces CBX7 antagonism towards WNT signalling. This effect was independent of EZH2 expression. Knock-out of EZH2 did not affect the ability of CBX7 to induce DKK-1. Treating CBX7-overexpressing breast epithelial or cancer cells with DKK-1 siRNA or DKK-1 inhibitor WAY-262611, rescues the TCF/LEF promoter activity and c-Myc expression (Kim, H. et al, 2015).

These findings indicate that proper WNT signalling is important for the PRC1 composition, since WNT signalling upregulates the expression of BMI1 (Galmozzi, E. et al, 2006; Yu, T. et al, 2012). BMI1 is more likely to be found in a complex together with CBX2 and CBX8 (O'Loghlen, A. et al, 2012). CBX2 and CBX8 are known to reduce the expression of CBX7 (O'Loghlen, A. et al, 2012). Since CBX7 negatively regulates WNT signalling by inducing DKK-1 (Kim, H. et al, 2015). It could be possible that besides the known WNT induction by PRC1 via ZIC inhibition (Chiacchiera, F. et al, 2016) also the WNT induction of BMI1 contributes to the increased WNT signalling due to the BMI related CBX2 and CBX8 induced CBX7 inhibition. If this is true, it would indicate that when Wnt signalling is low, the PRC1 complexes are more likely to contain CBX7 due to loss of Wnt induced BMI expression. The increase in CBX7 expression will lead to a reduction of CBX2, 4 and 8 expression (O'Loghlen, A. et al, 2012). This would also result in inhibition of WNT signalling (Chiacchiera, F. et al, 2016) and thereby reducing the already low WNT signalling even more. So the hypothesis deducted from PRC1 and WNT interactions is that when WNT signalling is low, this results in a PRC1 complex containing CBX7, which further lowers the effectiveness of WNT signalling.

4.0 How PRC1 and PRC2 can affect senescence via Wnt, TGF-β and BMP signalling.

As mentioned, Treatment of TGF-β1 and BMP2/4 has been shown to upregulate senescent cell markers, such as SA-β-gal (Li, Z. et al, 2016; Wu, J. et al, 2014; Buckley, S. et al, 2004; Pouliot, F. & Labrie, C. 2002). At the same time are BMI1 (PRC1 complex related) and EZH2 (PRC2 complex related) able to inhibit TGF-β1 and BMP signalling (Gargiulo, G. et al, 2013). Therefore it can be postulated that PRC1 complexes containing a BMI1 and PRC2 complexes containing EZH2 downregulate the TGF-β1 and BMP induced senescence. However, TGF-β1 is able to reduce the expression of EZH2, resulting in lower H3K27me3 of TGF-β target genes. This makes it easier for Smads to bind to TGF-β target genes, which makes the smad-dependent TGF-β pathway more effective (Cardenas, H. et al, 2016). Thus can TGF-β1 affect the effectiveness of PRC2. This might limit the effects of PRC1 as well, since PRC1 can

recognise the PRC2s catalysed H3K27me3 (Piunti, A., & Shilatifard, A. 2016). An indication for this could be a reduction in the amount of BMI1, however no study was performed to confirm this. Nevertheless, it seems plausible that the reduction of EZH2 due to TGF-β1 treatment helps in facilitating senescence. Upregulation of BMI1 and EZH2 might help in preventing senescence by reducing BMP and TGF-β1 induced senescence.

For Wnt signalling a similar hypothesis can be proposed. Wnt signalling and senescent cells have been associated in numerous ways. Firstly, Wnt signalling was found to be downregulated in senescent cells (Ye, X. et al, 2007). Low Wnt signalling has been shown to induce senescence (Ye, X. et al, 2007). On the other hand does prolonged treatment with high concentrations of Wnt agonists induce senescence as well (Zhang, D. Y. et al, 2011; Castilho, R. M. et al, 2009). This might however be contributed to stem cells being pushed out of their quiescent state, which contributes to the increase in senescence (Castilho, R. M. et al, 2009). Senescent cells secreted high amounts Wnt inhibitors and thereby keep the Wnt signalling low (Elzi D. J. et al, 2012).

Wnt signalling effects the composition of PRC1 by increasing the concentrations of BMI1 (Galmozzi, E. et al, 2006.) This might have a positive effect on Wnt signalling, since BMI1 is more likely to be found in a PRC1 complex together with CBX2 and CBX8. CBX2 and CBX8 downregulate the expression of CBX7. CBX7 is associated with an increase of DKK-1, which inhibits Wnt signalling (O'Loghlen, A. et al, 2012; Kim, H. et al, 2015), however this theory should be validated. It has been shown that PRC1 composition is changed when cells start to differentiate. CBX7 is replaced by CBX2, 4 or 8 and thereby facilitates differentiation. (Camahort, R, & Cowan, C. A. 2012; O'Loghlen, A. et al, 2012). Wnt signalling might contribute to the switching between PRC1 compositions, by increasing the BMI1 concentration. Thus induces Wnt signalling a PRC1 complex that favours BMI1, CBX2, 4 and 8, leading to differentiation (Camahort, R, & Cowan, C. A. 2012; O'Loghlen, A. et al, 2012). Since Wnt signalling is repressed by senescent cells it could be possible that the switch of changing CBX7 to CBX2, 4 or 8 is not induced. Which maintains and induces senescence.

This paper aimed to show connections between senescent cells and PRCs via TGF-β, BMP and Wnt signalling. It has been shown how TGF-β, BMP and Wnt signalling is affected by senescent cells and by the different PRC compositions (Cardenas, H. et al, 2016; Ye, X. et al, 2007). On the other hand, it was also shown that PRC composition can be altered due to TGF-β, BMP and Wnt signalling (Galmozzi, E. et al, 2006; Gargiulo, G. et al, 2013). The increased amounts of senescent cells in aged tissue effects TGF-β, BMP and Wnt signalling. These alterations in TGF-β, BMP and Wnt signalling lead to different PRC compositions (Camahort, R, & Cowan, C. A. 2012; O'Loghlen, A. et al, 2012). Different PRC compositions again affect TGF-β, BMP and Wnt signalling (Cardenas, H. et al, 2016; Ye, X. et al, 2007).

It seems that the increased amounts of senescent cells alter the balance in TGF-β, BMP and Wnt signalling in a way, which stimulates PRCs to facilitate senescence and thereby create an unwanted positive feedback loop. Disrupting this positive feedback loop might help in delaying the onset of age related disease.

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