Identification of Noncoding RNA Driving Epigenetic Changes During Cellular Senescence

Prof. Jian Yan Co-I: Prof. Jilin Zhang

Background of research

Ageing has become an urgent issue in developed societies due to problems arising from longer life expectancies while the societies lack of resources to address them. Cellular senescence is one of the hallmarks and driving force of tissue and organismal aging. Thus, people have been aiming to understand and find solutions to slowing down the progress of the adult cell senescence to maintain the functionality of various organs.

Regardless of either intracellular or extracellular senescence stimuli, one of the most defining features of cellular senescence is a stable cell cycle arrest in the G1 or G2 phase, preventing the aged cells from proliferation [1]. The cell cycle arrest by cellular senescence is primarily dictated by the p53/p21^{CIP1} and p16^{INK4A}/RB Cyclin Dependent Kinase inhibitor (CDKI) pathways, whose depletion could lead to bypass of the cellular senescence [2, 3]. By manipulating these senescence-associated factors, ageing-associated cell senescence could be reversed and fitness could be improved. For example, the Nobel Laureate Prof Shinya Yamanaka and others have shown that the p53-p21 pathway serves as a barrier in induced pluripotent stem (iPS) cell generation [4, 5]. Consistently, another study revealed that blockade of senescence-associated microRNA-195 in aged skeletal muscle cells facilitates reprogramming to produce iPS cells [6]. Furthermore, multiple *in vivo* studies have demonstrated that clearance of p16I^{NK4A}-positive cells could significantly attenuate ageing-related dysfunction of multiple organs without observing any overt side effects [7, 8].

Alteration in epigenetic modifications is prevalent in senescent cells as a result of accumulating metabolites and mutations [9]. For example, senescent hematopoietic stem cells show elevated levels of α-ketoglutarate, an essential co-factor of the ten-eleven translocation 2 (TET2) enzyme, and reduced S-adenosylmethionine, a methyl-group donor, both likely causing an ageing-associated reduction of DNA methylation, which are essential for the pluripotency and self-renewal of stem cells [10-12]. Adult stem cells display global hypomethylation compared to young stem cells [13, 14]. Besides DNA methylation, changes of histone modifications are also pervasive in senescent cells. For example, histone H3K4me3 is important for priming gene expression during cellular differentiation and it was recently found to be increased along senescence of hematopoietic stem cells [15]. Deregulated repressive histone markers also play essential roles in driving the cellular senescence. For instance, subunits of Polycomb group (PcG) complex proteins PRC1 and PRC2, including BMI1, MEL18, CBX4, CBX7, CBX8, EZH1/2, and SUZ12, are downregulated, causing loss of H3K27me3 at the *INK4a/ARF/INK4b* locus, and consequent upregulation of p16^{INK4A} [16]. Another example showed in muscle that the number of repressive histone marks such as histone H3 lysine 9 trimethylation (H3K9me3) and H3K27me3 tended to increase in old stem cells [17], causing a progressive intensification of heterochromatin and loss of stem cellular plasticity during ageing. Therefore, it is imperative to elucidate epigenetic disruptions in cellular senescence and investigate the underlying mechanisms of how the alternations drive aberrant gene expression and senescence.

The role of long noncoding RNA (lncRNA) in epigenetic regulation of gene transcription has been increasingly revealed. For instance, lncRNA HOTAIR mediated transcriptional silencing of the *HOXD* gene locus through direct interaction with PRC2 complex and recruitment of EZH2 (the catalytic subunit of PRC2) for deposition of H3K27me3, providing the first piece of evidence that lncRNA can be directly implicated in remodeling the chromatin epigenetic environment [18]. More and more lncRNAs, such as XIST, H19, MALAT1, or KCNQ1OT1, have been identified as epigenetic co-regulators, contributing to epigenetic regulation of transcription [19]. NEAT1 (nuclear-enriched abundant transcript 1) is an essential RNA component of nuclear paraspeckles, involved in transcriptional regulation. It is reported that NEAT1 can regulate the neural stem cell (NSC) differentiation and migration by activating the Wnt/β-catenin pathway [20]. Interestingly,

NEAT1 acts as a scaffold molecule to recruit EZH2 to catalyze H3K27-specific methylation and repress the expression of CTNNBIP1, GSK3B, and Axin2, thereby promoting the nuclear transport of β-catenin [21]. The result revealed an active role of lncRNA in mediating deposition of epigenetic modifications and gene expression, conferring impact on stem cell pluripotency and self-renewal. Up to 20% of human lncRNAs could be associated with epigenetic modifiers, underscoring the influence of lncRNA on epigenetic modifications [22].

The lncRNA is also involved in the progress of cellular senescence. When depleting the lncRNA Zeb2-NAT, old fibroblasts re-gained a potential capacity for reprogramming similar to that of a young cell [23]. Furthermore, lncRNA PANDA facilitates recruitment of PRC complex to repress transcription of $p16^{INK4a}$ gene in proliferating cells. When PANDA is depleted, cellular senescence would be promoted [24]. Similarly, the antisense lncRNA for $p16^{INK4a}$, named ANRIL, recruits PRC1 and PRC2 complexes to the $p16^{INK4a}$ promoter and represses its transcription, which consequently releases cell from cell cycle arrest and allows progression of mitosis. ANRIL is downregulated in senescent cells [25]. There is thus a compelling need to further understand how lncRNA functions in cellular senescence by modulating epigenetic modifications. It is also plausible to speculate that, by targeting certain lncRNAs, cellular rejuvenation might be achieved through dampening the senescence-associated epigenetic alterations.

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Fig. 1 | Development of Chrom-seq to identify H3K27me3-associated RNAs.

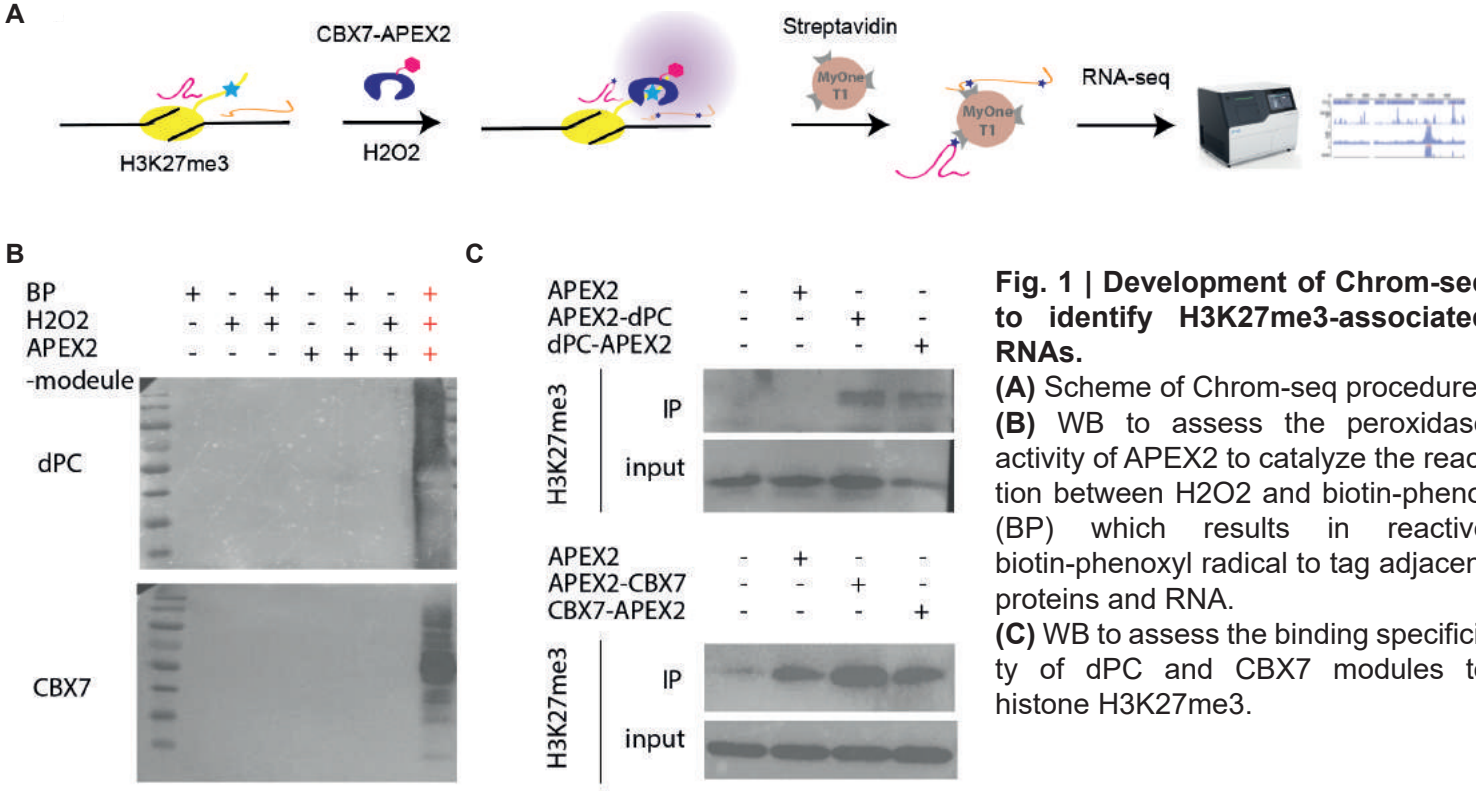

Fig. 1 | Development of Chrom-seq to identify H3K27me3-associated RNAs.
(A) Scheme of Chrom-seq procedure.
(B) WB to assess the peroxidase activity of APEX2 to catalyze the reaction between H2O2 and biotin-phenol (BP) which results in reactive biotin-phenoxyl radical to tag adjacent proteins and RNA.
(C) WB to assess the binding specificity of dPC and CBX7 modules to histone H3K27me3.

Fig. 2 | Indetification of senescence-associated RNAs.

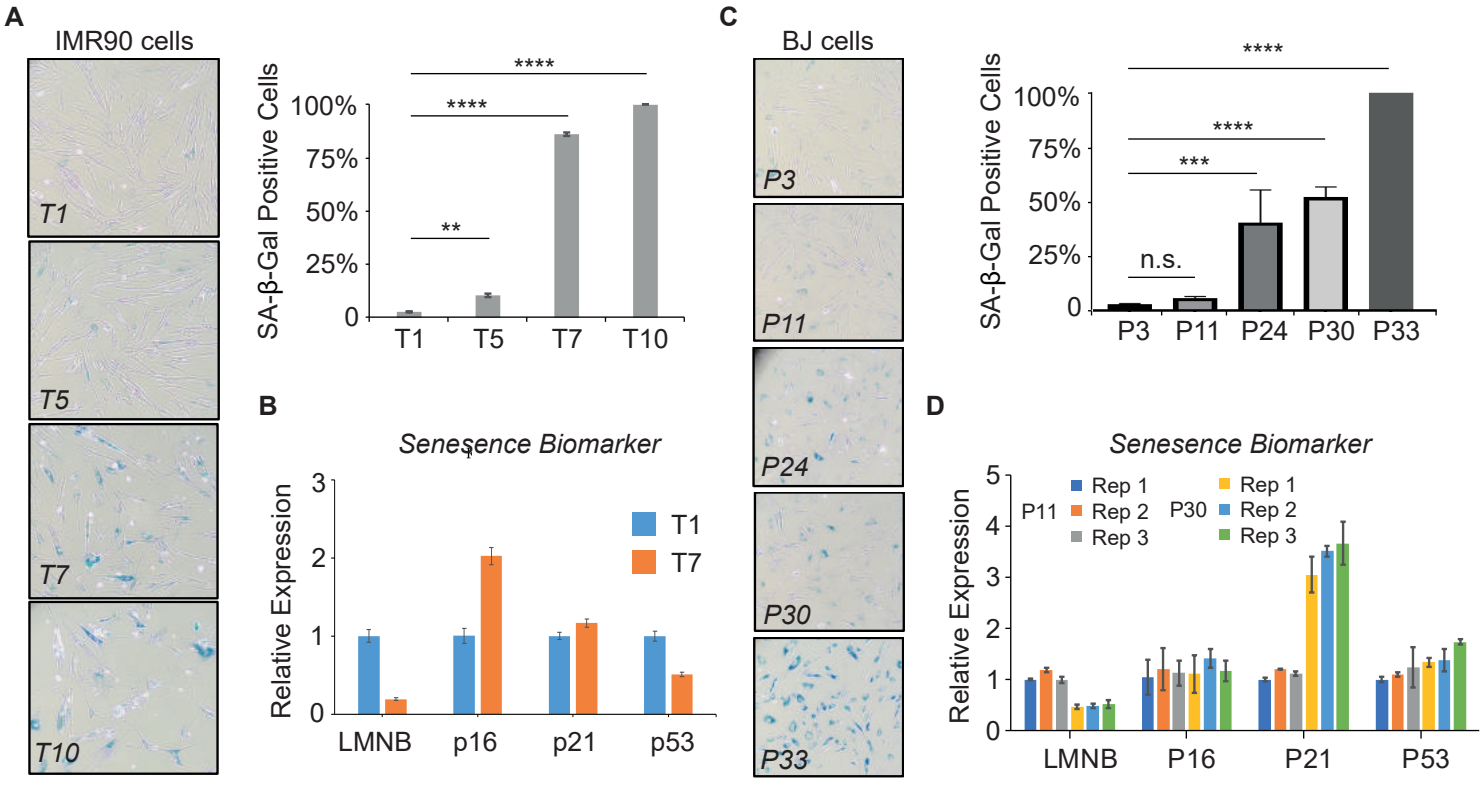

Fig. 2 | Indetification of senescence-associated RNAs.
(**A**) Senescence-associated-β-Gal staing of IMR90 cells. The blue-positive staining indicates the senescence of the cells. The inset bar plot quantifies the proportion of blue-positive cells.
(**B**) qPCR results show the change of expression of senescence biomarker genes between T1 and T7 passages of IMR90 cells, including Lamin B1 (LMNB), p16, p21 and p53. Three replicative experiments are included to caculate the mean and standardard errors (indicated by error bars).
(**C**) Senescence-associated-β-Gal staing of BJ cells.
(**D**) qPCR results show the change of expression of senescence biomarker genes between P11 and P30 passages of BJ cells. Three replicative experiments are included to caculate the mean and standardard errors (indicated by error bars).