Synopsis

Cellular senescence has been proposed to be equivalent to organismal aging and is one of the outcomes of the cell fate decision process in response to DNA damage that occurs in cells. When a cell encounters DNA damage, the cell cycle is immediately halted to evaluate which decision to take in response to genomic insult. The choices are between repairing the damage and continue division, or enter a non-replicative but viable state called senescence or to die if damage is severe (Figure 1). The signaling cascade, which detects this damage and regulates the cell fate decision, is collectively called as DNA damage response (DDR). However, the exact mechanism of how delineation for each decision happens is still not clear. Since DNA damage works as a mediator for cell fate decision, my work aimed to study senescence as a DNA damage response.

In addition, the role of free radicals like ROS in cellular senescence is not very clear because though an increase in their concentrations is recorded in aged cells, it is not evident if the increase seen the cause or the effect of aging, primarily because they themselves capable of causing DNA damage. This conundrum have always led to confounding observations wrt role of free radicals in the cellular senescence process and if the senescence is caused through agents which rely on ROS to cause DNA damage, ROS becomes absolutely integral to the aging process. To understand this aspect formed the first line of investigation in my work along with identification of the sensor of DNA damage, which drives various cell fates.

During organismal ageing there is an accumulation of senescent cells, which could be the major reason for functional decline of tissues and organs with age. However, to study changes associated with signaling molecules with respect to ageing, a cellular model system for senescence driven through DNA damage was needed, using which interplay between senescent / aged cells and cellular niche can be established. Studying the spatial and temporal alterations in signaling dynamics, within the cell as well as with the neighbouring niche during the senescence process in anticipated to provide us better understanding about the complex process of ageing. For this, the objectives were defined to establish and characterize the DNA damage induced senescence model using various parameters, and especially study the signaling dynamics of GPCR mediated...
signaling in senescence. The role of chemokine receptor, CXCR4 and its ligand, CXCL12 mediated signaling was chosen for the study.

The following sections describe the findings that were obtained from the various objectives studied during the course of this study.

**Section 1. Development and characterization a model system to study cellular senescence as a DNA damage response.**

In this part of the study, I characterized genotoxic stress induced cellular senescence model using 5-Bromodeoxyuridine as the DNA damaging agent. BrdU, owing to its property of being a thymidine analogue, is incorporated in dividing cells, and this incorporation is recognized as DNA damage. This triggers ‘persistent’ DNA damage response signaling, including activation of ATM kinase, one of the primary DNA damage sensor. As anticipated, the DDR response detected was directly proportional to the dose of BrdU treatment and so was Reactive Oxygen Species (ROS) levels, a known senescence mediator.

Using this model system of direct DNA damage mediated DDR activation and induction of cellular senescence, the growth-arrested cells were extensively characterized for presence and quantum of most of the senescence associated markers known in literature. BrdU treated cells, which became senescent showed presence of DNA damage, morphological changes like flat, enlarged, granule rich appearance, expression of senescence associated molecular markers like p21, IL8, showed senescence associated beta galactosidase activity, refractiveness to growth factor for division, increased ROS levels, Golgi dispersion, etc. The secretome of the treated cells also showed increased secretion of inflammatory cytokines which are attributed to a senescence phenotype, called as Senescence Associated Secretory Phenotype (SASP), which triggered proliferative and migratory effect on cancer cells. Overall, in this part of the study, it was established that BrdU can cause DNA damage and induce senescence as one of the cell fate in response to the intermediate dose of damage. The senescent cells generated in the model system was established to be akin to senescence observed by replicative exhaustion of normal cells, thereby making our model applicable to the physiological studies as well.
Section 2. Insights into the role of ATM-ROS axis during senescence initiation and maintenance using DDR mediated cellular senescence model.

While the BrdU model system for generating senescent cells was being developed and characterized, it was observed that there is an increase in ATM activation as well as ROS production concomitant to the a dose of BrdU. At the same time it was also observed that senescent cells showed persistent DDR signaling and high levels of ROS. Using this premise, in the second objective of my study I aimed to identify if ATM and ROS are critical during initiation of senescence, when the cells are insulted with the DNA damaging agent or during the maintenance of senescent state of the cells. By quenching ROS during the initiation state, I recorded that ROS is not critical for inducing senescence and perhaps the increase in ROS levels in senescent cells is due to their higher metabolic activity.

By inhibiting ATM activation during DNA damage, it was observed that BrdU induces senescence through direct DNA damage, and active ATM and DDR signaling is absolutely critical for the senescence initiation. It was also established that ATM is not just a DNA damage sensor but also a redox regulator in the senescence model system. Prevention of ATM activation in presence of DNA damage blocked senescence initiation and also triggered increased ROS levels in the cells affecting their long term viability, suggesting ATM regulates ROS levels as well in addition to sensing DNA damage.

In order to study the role of ATM-ROS axis in the maintenance of senescence state, already senescent cells were subjected to ROS quenching and/ or ATM inhibition and it was identified that both these signaling molecules are essential for maintaining the viability of senescent cells. The findings from these study thereby show that senescence can be divided into two temporally distinct stages, initiation or early senescence stage and second, maintenance stage of senescence. Overall, I was able to characterize the presence of temporally linked ROS – dependent and ROS – independent events in cellular senescence, which are independently mediated by ATM kinase (Figure 1).
Cells enter senescence state in response to DNA damage, depending on the dose of insult, through an ATM dependent and ROS independent pathway. Unlike this ATM-ROS axis is critical for the maintenance of senescent state of damaged but viable cells.

Section 3. Understanding the role of CXCR4 – CXCL12 mediated signaling in senescence.

Age dependent changes in cellular signaling are less explored and I was specifically interested in understanding how presence of senescent cells affects its microenvironment or vice versa i.e. how microenvironment affects senescent cells. In this premise the third objective of this study was defined towards identifying role of a GPCR, CXCR4 mediated signaling in cellular senescence and associated inflammation. CXCR4 is a ubiquitously expressed GPCR and it’s only known ligand is CXCL12/SDF1α (stromal derived factor α), which is a homeostatic chemokine (i.e. its levels does not change under most physiological conditions). During characterization of DNA
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damage induced senescence model system, it was observed that this receptor expression is induced during DNA damage cells, which was also found to be so from data available from other gene expression studies as well.

During the course of my work, I identified that senescent cells show CXCR4 up regulation in response to DNA damage, mediated through activation of ATM kinase - HIF1α axis and plays a critical role in enhancing the senescence associated inflammatory response in presence of its ligand, CXCL12. This CXCL12 dependent enhanced inflammatory response in damaged cells was determined to be sensitive to the pertussis toxin treatment and hence dependent on G protein activation. Further downstream analysis revealed the pro-inflammatory effect of the CXCR4 receptor activation was due to cAMP level suppression post activation by the Gαi subunit. Given that cAMP levels are antagonistic to inflammatory phenotype, using a library of pharmacological compound library, I also discovered that cAMP specific PDE, phosphodiesterase 4A, is also involved in regulating inflammatory response during the initiation stage of cellular senescence. The screen also confirmed the involvement of previously identified molecular components such as p38 MAPK and leukotrienes in the senescence associated inflammatory phenotype.

The examination of the role of the CXCR4- CXCL12 axis in the deeply senescent cells surprisingly revealed that deeply senescent cells are refractory to CXCL12 stimulation in terms of inflammatory response, which was experimentally determined to be associated with impaired calcium release.

Overall, the findings from this part of the study revealed a novel signaling cascade where CXCR4 up regulation is a part of the DDR response in cells, which utilizes the Local Excitation Global Inhibition (LEGI) mechanism to enhance the sensitivity of the damaged cells to its ligand CXCL12. This enhanced sensitivity mediates the CXCL12 dependent inflammatory response, which aids in attracting immune cells for clearance of these damaged cells. Once the cells have entered the senescent state, the axis is physiologically down modulated and the senescent cells showed refractiveness to CXCL12 stimulation, probably to prevent persistent acute inflammation, if the senescent cells are not cleared (Figure 2).
Figure 2. CXCL12-CXCR4 axis in cellular senescence. During senescence initiation stage, when cells encounter DNA damage (Step 1), there is induction of CXCR4 receptor (Step 2), which enhances of CXCL12 mediated signaling for increased inflammatory response (Step 3). In the maintenance stage, where the cells are not cleared (Step 4), the axis is suppressed (Step 4), thereby bringing the levels of inflammatory secretome down, and thereby preventing damage to the cells (Step 5).