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# Is Cellular Senescence of Dopaminergic Neurons the Cause of Local Inflammation in the Midbrain Observed in Parkinson's Disease?

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Current research investigating the pathomechanisms of neurodegenerative disorders of the central nervous system (CNS), such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), or Parkinson's disease (PD), led to the understanding that these diseases have to be seen in the context of immune responses [1]. In other words, inflammation plays a central role in neurodegenerative disorders of the CNS. The ultimate question whether the immune responses that are observed in these diseases are causative or consequential, is debated. However, increasing evidence suggests that activation of both the adaptive and the innate immune system can drive the disease progression. One exemplary disease, in which both the adaptive and the innate immune system have been shown to be involved, is PD. The classical motor symptoms observed in PD patients are caused by the loss of dopaminergic (DA) neurons of the substantia nigra pars compacta (SNpc) [2-5]. DA neurons of the SNpc are thought to be particularly vulnerable because of their high levels of reactive dopamine, high energy demand and mitochondrial turnover, calcium handling, as well as their large axonal arborizations [6-8]. Although many causative gene mutations in familial forms have been analyzed, the underlying cause of sporadic PD is not understood. Interestingly, activated innate immune cells of the CNS, microglia, have been shown to directly contribute to the death of DA neurons in the midbrain [9]. Reactive microglia have been reported to be particularly abundant in the midbrain [10]. In addition, activated cells of the adaptive immune system, T lymphocytes, have also been observed in postmortem PD brain tissues [11]. These activated T cells seen in sporadic PD have furthermore been shown to actively kill DA neurons via the Interleukin 17 (IL-17) pathway. In summary, there is convincing evidence that immune response in the midbrain contributes directly to the loss of DA neurons in the SNpc. The biggest question that remains is: what causes this detrimental immune (over-)reaction? It has recently been reported that DA neurons are able to present alpha-synuclein as antigen causing activation of T cells. Why are T cells and microglia so abundant in the SNpc even before symptoms occur?

Recently, we discovered that post-mitotic DA neurons can become senescent. We have identified Special AT-Rich Sequence-Binding Protein 1 (SATB1) as a genetic master regulator that plays a neuroprotective role specifically in DA neurons of the SNpc [12]. Importantly, *SATB1* was identified as a genetic risk factor for PD [13,14]. In summary, we sought to characterize the function of this transcriptional repressor in human DA neurons. When we knocked out *SATB1* in pure cultures of human embryonic stem cell-derived DA neurons, we found that these cells first differentiate normally, but when they reach a mature state in which autonomous pacemaker activity starts, they become senescent [15]. Interestingly, stem cell-derived cortical (CTX) neurons were not affected by the loss of *SATB1* and no differences between knockout (KO) and wild-type (WT) were observed. The senescence phenotype that was observed in both human SATB1-KO as well as mouse Satb1-knockdown DA neurons resembled every aspect of the phenotype typically described in mitotic cells. In the SATB1-KO DA neurons, we observed classical senescence hallmarks such as dysfunctional and swollen mitochondria, lysosomal dysfunction, elevated levels of reactive oxygen species (ROS) as well as oxidized proteins, lipofuscin accumulation, senescence associated β-galactosidase (SA

β-gal) activity, increase of nuclear diameter in correlation with decreased lamin B1 expression, and activation of the senescence associated secretory phenotype (SASP). The SASP is characterized by the release of pro-inflammatory factors that cause a local inflammation in the surrounding tissue and recruits and activates immune cells detecting and removing senescent cells [16]. This mechanism is central to the immune response to senescent cells. Interestingly, we observed that senescent DA neurons in pure cultures persist, whereas senescent DA neurons *in vivo* trigger immune response and recruit microglia that actively remove the senescent DA neurons (Figure 1A) [15]. It is likely that the SASP is also attracting and activating T cells which could potentially kill senescent DA neurons. Re-assessment of the expression profile changes of the human ESC-derived DA neurons revealed a highly significant upregulation of the cytokine CCL2, the interleukin receptor IL17-R, and some major histocompatibility complex genes (MHC) such as HLA-B and HLA-C. It has previously been reported that CCL2 attracts T cells [17]. Moreover, it was proposed that T cells can actively kill DA neurons in PD patients via the IL17 pathway - specifically DA neurons which express IL17-R [18]. Our findings therefore implicate that senescent DA neurons actively trigger an immune response and attract immune cells such as T cells and microglia. Moreover, upregulation of receptors, such as IL17-R, actively mediate the removal of these cells. Given that alpha-synuclein accumulation has been shown to trigger a DNA damage response [19], it is very likely that there is an age-related increase in the number of DA neurons that express a program of cellular senescence which is induced by the activation of the DNA damage response. Occurrence of senescent cells in the SNpc would trigger a local inflammation and attract and activate both innate and adaptive immune cells which would actively remove the senescent DA neurons. This theory is in line with the finding of unusually high numbers of both microglia as well as T cells present in the SNpc of PD patients. This increase of immune cells occurs before the onset of symptoms of PD [20]. Even in healthy aging, there is a constant loss of nigral neurons throughout life, with an estimated ~10% loss in every decade of life starting around the age of twenty [21]. Since the DA neurons are particularly prone to senesce, we hypothesize that there is a constant turnover of cells that become senescent. These senescent cells are removed by an intact immune system. With age, it is thought that immune cells get more used to senescent cells and are not detected and removed as efficiently any more [22]. It is easily imaginable that if senescent cells remain in the midbrain, they will cause and sustain a local inflammation which can be detrimental for the present DA neurons. The SASP will trigger an inflammatory response and attract and activate immune cells (summarized in Figure 1A). Our RNA-Seq data from senescent human SATB1-KO DA neurons revealed

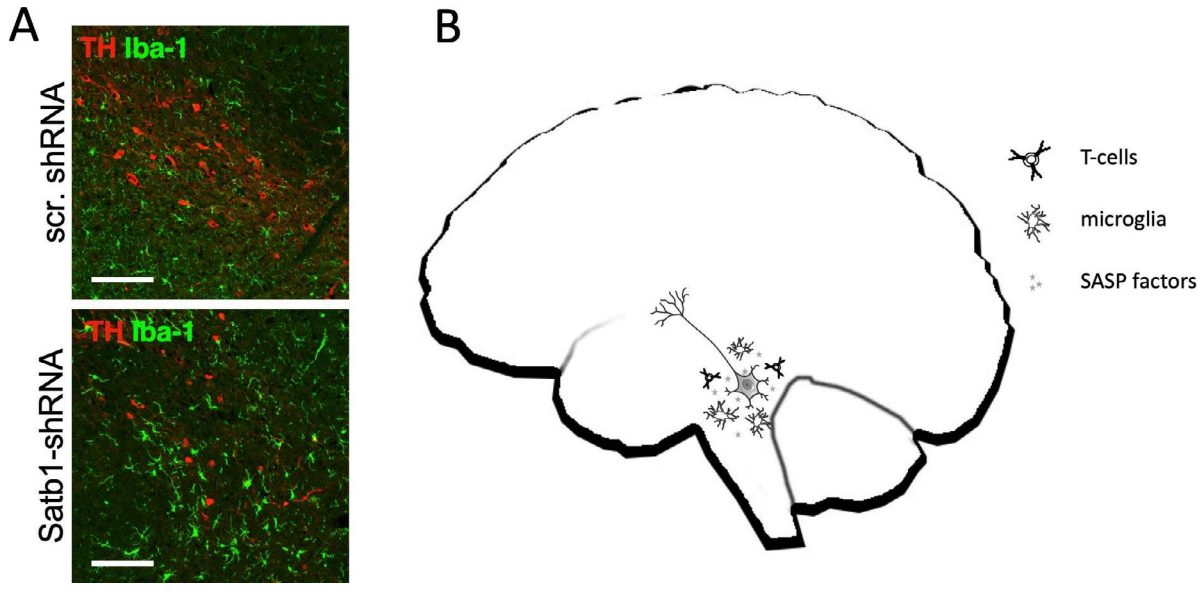


**Figure 1: Senescence of dopaminergic neurons in the *substantia nigra pars compacta*. A)** Representative confocal images of mouse SNpc: tyrosine hydroxylase (TH) and Iba-1 staining was performed two weeks after virus injection expressing scrambled (scr.) shRNA and *Satb1* shRNA. Note the abundance of highly activated microglia (green) located at the senescent DA neurons (red) and colocalization of Iba-1 and TH signals in the SNpc under Satb1-knockdown. Microglia appear to attack intact DA neurons [15]. Scale bars: 100 μm. **B)** Potential immune reactions in the human brain: Senescent dopamine neurons secrete inflammatory factors that attract and activate immune cells such as microglia and T cells. These immune cells can actively attack neurons and kill them.

a significant upregulation of SASP/immune genes that potentially attract immune cells. CCL2 was ~6-fold upregulated, but was not significantly altered in SATB1-KO CTX neurons (Figure 2A). CCL2 has been shown to attract T cells and could explain the high abundance of these immune cells in the SNpc of PD patients. Once in close proximity, T cells have been shown to kill DA neurons via the IL17 pathway. Interestingly, senescent DA neurons express significantly upregulated levels of IL17R, a gene that mediates neuronal cell death by IL-17–IL-17R signaling and activation of Nuclear Factor Kappa B (NFκB) (Figure 2B) [18]. In line with the discovery that antigen presentation of DA neurons is critical for the immune response [23], we found significantly elevated expression of Major Histocompatibility Complex (MHC) class I genes such as *HLA-B* and *HLA-C* which were not significantly changed in CTX SATB1-KO neurons (Figure 2C and 2D). In summary, there is striking evidence that DA neurons are particularly prone to senesce and that the presence of the SASP in the SNpc could potentially explain the occurrence of a local inflammation which in turn triggers a massive immune response and finally a removal of DA neurons. Importantly, we have previously demonstrated that senescent DA neurons react to senolytics treatment and can be killed by this new class of compounds. Given that senescent DA neurons seem to occur sporadically, it would potentially be feasible to interfere with a senolytics treatment to ameliorate the local inflammation and most importantly, to prevent spreading of senescence which could lead to dramatic loss of DA neurons and consequently to PD.

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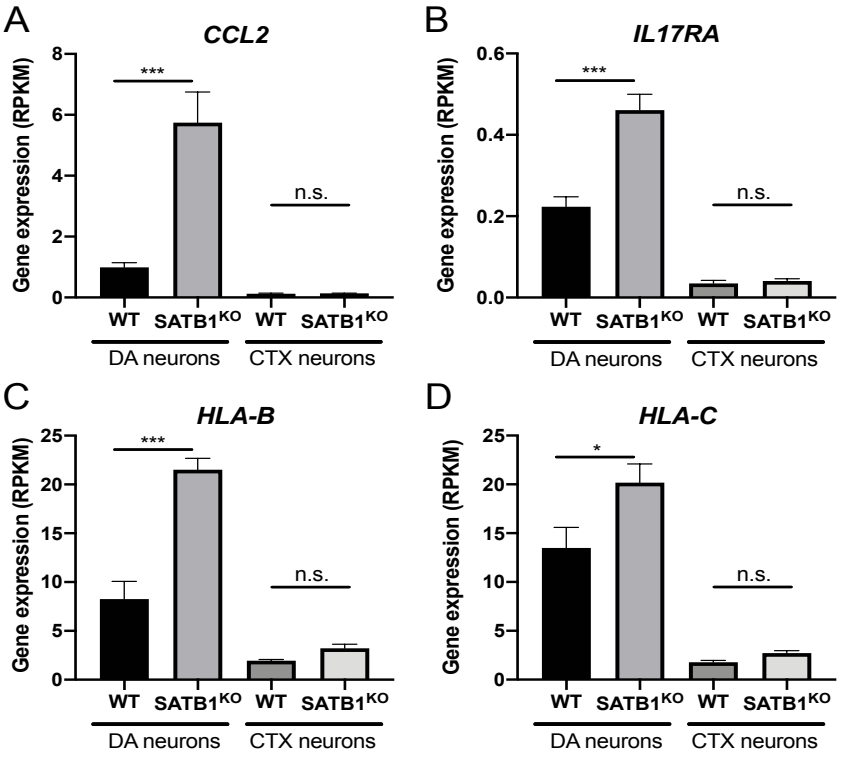


**Figure 2: Molecules that attract immune cells and mediate cell removal are upregulated in senescent dopamine neurons. A)** CCL2 attracts T cells, **B)** IL17RA mediates cell death by binding to its ligand IL-17 which is expressed in T cells, **C)** HLA-B and **D)** HLA-C are important for antigen presentation. All presented genes were significantly upregulated in senescent (SATB1- KO) DA neurons but not altered in cortical neurons. Student's t-test was performed for significance (* = $p<0.05$, *** = $p<0.001$, n.s. = not significant). Data presented as mean ± standard error of the mean. RPKM = Reads Per Kilobase of transcript, per Million mapped reads. Raw data available under the accession number *ArrayExpress*: E-MTAB-5965 [15].

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