A novel redox cycle diverts cells from oncogene-induced senescence into cancer

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Igelmann et al. **report a novel metabolic cycle, which they name HTC, that converts NADH into the key antioxidant factor NADPH. The HTC is repressed by the tumor suppressors p53 and RB, and this determines whether oncogene-expressing cells undergo senescence (HTC^{off}) or malignant transformation (HTC^{on}).**

A common consequence of oncogenic signaling in normal cells is mitochondrial dysfunction, the production of high levels of reactive oxygen species (ROS), and activation of the cellular senescence program. In this manner, oncogene-driven proliferation comes to a halt and oncogene-induced senescence (OIS) prevents the acquisition of malignant properties, acting as a potent tumor suppressive response (*Ou et al., 2020*). In the search to understand the metabolic features of senescent cells, *Igelmann et al., 2021* have discovered a novel pathway to generate NADPH, the most important and limiting source of reductive power for anti-oxidant defenses. This new route consists of three coupled reactions within a ternary complex formed by malate dehydrogenase1 (MDH1), malic enzyme 1 (ME1), and pyruvate carboxylase (PC) (**Figure 1**). The first two reactions involve the transfer of a hydrogen anion (or hydride) from NADH to oxaloacetate, thus generating malate, and then from malate to $NADP^+$ to finally produce NADPH. The third reaction closes the cycle by regenerating oxaloacetate. The authors have appropriately named this cycle as the hydride transfer complex (HTC). The genes encoding these three enzymes are transcriptionally repressed by both p53 and RB, two of the most important tumor suppressors. Accordingly, oncogene-expressing cells with functional p53 and RB pathways repress the HTC and cannot efficiently produce NADPH. This renders these cells unable to counteract ROS-induced oxidative damage and undergo senescence (**Figure 1**). In contrast, oncogene-expressing cells deficient for p53 or RB, upregulate the HTC, which allows them to generate abundant NADPH and, thus, cope with high levels of oxidative damage and continue their path toward malignant transformation (**Figure 1**). These findings are in line and expand an earlier report demonstrating the importance of NADPH and malic enzyme repression by p53 for tumor suppression (*Jiang et al., 2013*).

Cellular senescence, induced by oncogenes or by other types of stressors, for example, chemotherapeutic drugs, is characterized by high levels of NADH (also referred to as low NAD^+/ NADH ratio) (*Chapman et al., 2019; Wiley et al., 2016*). This

is a manifestation of the mitochondrial dysfunction caused by senescence-inducing stimuli, although the exact details about why NADH levels increase in senescent cells were poorly defined. The current work by *Igelmann et al.* identifies the partial loss of Complex I subunits of the electron transport chain (ETC) as a key event mediating cellular senescence (*Igelmann et al., 2021*). Complex I is the entry point of NADH-derived electrons into the ETC and, therefore, an inefficient Complex I activity readily explains why senescent cells accumulate such high levels of NADH. In an elegant experiment, the authors show that overexpression of the yeast single subunit Complex I (NDI1) is sufficient to prevent senescence in cells experiencing mitochondrial damage. At this point, it is important to remind that cytosolic anti-oxidant defenses rely mostly on NADPH rather than NADH (Chandel, 2021). In other words, the reductive power of NADH accumulated in oncogene-expressing cells would need to be converted into NADPH to counteract the oxidative damage exerted by ROS. Normal cells use the NAD^+ kinase (NADK) to generate $NADP^+$, which is then converted into NADPH by various dehydrogenases, including ME1 (*Rather et al., 2021*). However, the NADK route to NADPH starts with NAD^+ and, as we have mentioned, senescent cells have low NAD^+/NADH ratios. Therefore, senescent cells are locked in a state of redox imbalance characterized by high NADH that cannot be converted into NADPH, and as a consequence ROS-induced oxidative damage remains unabated.

A key observation of *Igelmann et al.* is that the accumulation of NADH in cells undergoing mitochondrial damage requires functional p53 and RB pathways (*Igelmann et al., 2021*). This was the starting point to discover that p53 and RB transcriptionally repress the three enzymes that act sequentially in the HTC cycle and whose net result is the conversion of NADH into NADPH (**Figure 1**). In cells lacking functional p53 or RB, the HTC is fully active, thereby consuming NADH and generating NADPH, and this explains why mitochondrial damage in these cells does not result in accumulation of NADH. It is interesting to note that the HTC cycle occurs in the cytoplasm and, while malate dehydrogenase 1 (MDH1) and malic enzyme 1 (ME1) are indeed cytosolic proteins, pyruvate carboxylase (PC) is widely considered a mitochondrial enzyme. The authors use an array of techniques including fluorescent microscopy, immunogold staining, co-immunoprecipitation, and proximity ligation assays (PLA) to demonstrate that, in cells undergoing oncogenic stress, a fraction of PC resides in the cytoplasm where it is part of the HTC. The authors go even farther and demonstrate that purified proteins of the HTC spontaneously assemble *in vitro* into a ternary complex and that in cancer cells overexpressing these three proteins, the HTC enters into phase-separated bodies in the cytosol (*Igelmann et al., 2021*). As mentioned above, all three enzymes of the HTC are transcriptionally repressed by p53 or RB. Accordingly, senescent cells present low levels of the HTC, whereas cancer cells present high levels. Moreover, using metabolic flux analysis, the authors demonstrate that forced simul taneous overexpression of the three enzymes of the HTC diverts pyruvate toward the HTC, and that this is sufficient to increase NADPH levels at the expense of NADH. This culminates with the demonstration that mitochondrial dysfunction in cells with forced expression of the HTC does not result in low NAD^+/NADH ratios. Importanlty, these cells have high levels of NADPH, present low ROS damage, and do no undergo senescence.

Given the ability of the HTC to prevent senescence, *Igelmann et al.* reasoned that high levels of the HTC should render cells prone to oncogenic transformation. Indeed,

simultaneous overexpression of the three enzymes that constitute the HTC allowed direct neoplastic transformation of mouse embryonic fibroblasts (MEFs) by oncogenic Ras without the need to concomittantly inactivate p53 or RB (*Igelmann et al., 2021*). The authors further hypothesized that the ability of the HTC to sustain tumorigenesis might be of particular relevance in cancers that use lactate as a carbon source, such as prostate cancer. Cancer cells dependent on lactate are forced to convert lactate into pyruvate, a reaction that consumes NAD^+ and generates NADH. Interestingly, the authors found high levels of each of the three HTC components in up to 60% of human prostate adenocarcinomas by immunohistochemistry. Moreover, by using PLA in a mouse model of prostate cancer, they found that MDH1, ME1, and PC assemble in the cytoplasm of cancer cells but not in the cytoplasm of neighboring non-cancer cells (*Igelmann et al., 2021*).

In summary, *Igelmann et al*. discover a novel metabolic cycle, the HTC, that can transfer the reducing power of NADH to NADPH and demonstrate that transcriptional repression of this cycle by p53 and RB is an important tumor suppressive mechanism (*Igelmann et al., 2021*). Pharmacological targeting of the HTC could be an exciting approach for the development of novel senescence-inducing cancer therapies.

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DECLARATION OF INTERESTS

M.S. is a founder, shareholder, and advisor of Senolytic Therapeutics, Inc., Iduna Therapeutics, Inc, and RejuverSen, AG.

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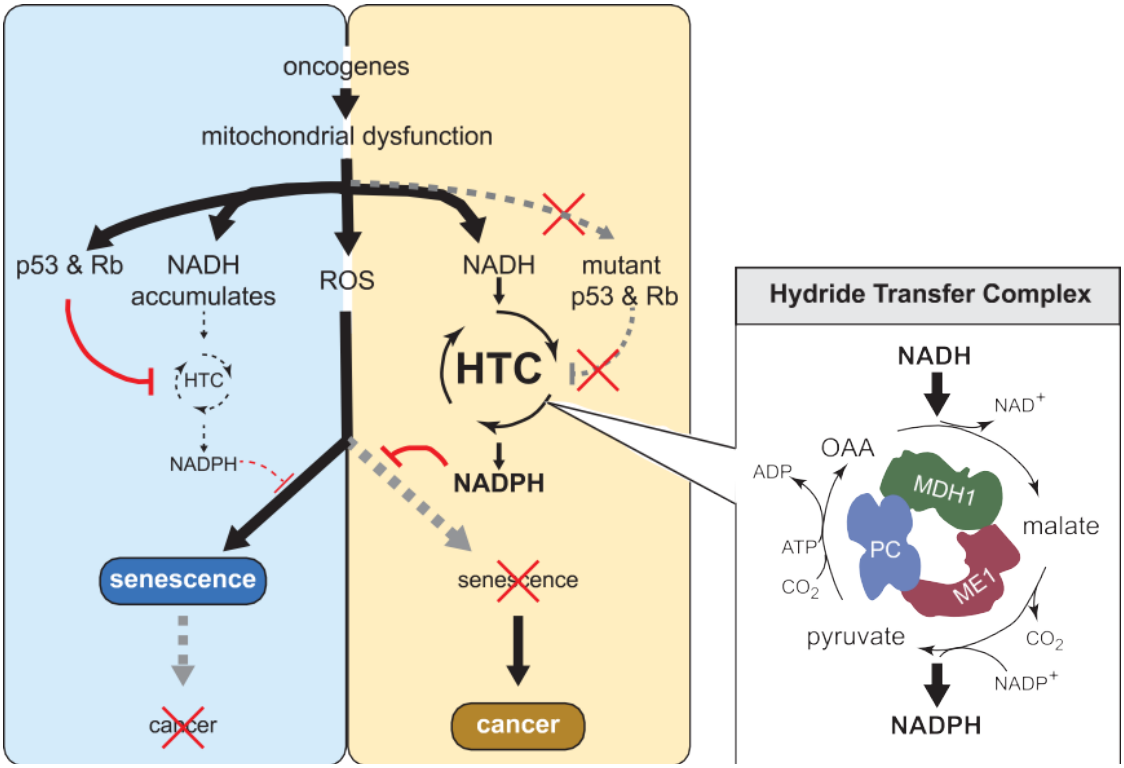

Figure 1. The hydride transfer complex (HTC) can convert the reducing power of NADH into NADPH, providing p53- or RB-deficient cancer cells with antioxidant protection to bypass oncogene-induced senescence

Cells overexpressing oncogenes undergo senescence due to mitochondrial dysfunction that causes elevated levels of ROS and NADH in a p53- and RB-dependent manner. *Igelmann et al.* show that a crucial function of p53 and RB in oncogene-induced senescence (OIS) is the suppression of a metabolic cycle named HTC (blue box). The HTC is composed of malate dehydrogenase 1 (MDH1), malic enzyme 1 (ME1) and pyruvate carboxylase (PC) (white box). This cycle transfers hydrides (H^-) from NADH to $NADP^+$. The HTC, by consuming NADH and generating NADPH, restores the redox balance and at the same time fuels antioxidant systems with their essential cofactor, NADPH. When p53 or RB become active, the expression of the three HTC enzymes is transcriptionally repressed, and this results in insufficient NADPH levels to counteract ROS-dependent damage, thereby, leading to OIS. Cancer cells with deficient p53 or RB ex- press high levels of the HTC enzymes and thereby efficiently convert NADH into NADPH, maintaining the redox balance and neutralizing ROS-induced damage (yellow box). As a consequence, these cells do not undergo senescence, despite mitochondrial dysfunction and continue proliferating toward malignant transformation and cancer.