**////Title: How Cancer Cells Overcome the Obstacle of Senescence**

**////Stand-first**:

Cellular senescence [suh-NEH-Sns] is the process by which cells age and permanently stop dividing but do not die. While the process of senescence creates a barrier to tumour formation, it can still be overcome by cancer cells. Sebastian Igelmann, a PhD student supervised by group leader Dr Gerardo Ferbeyre at the University of Montreal, has identified a group of enzymes that work together to reprogramme cellular metabolism. This work provides important insight into how tumour cells may initiate proliferation and circumvent senescence. Critically, this specialist group of enzymes provides a potential therapeutic target for human cancer treatment.

**////Body text:**

Senescence is a mechanism through which the development of tumours may be suppressed, thus preventing the initiation of cancer. Senescent [suh-NEH-Snt] cells expressing oncogenic [ong-kow-JEH-nuhk] mutations cannot divide and replicate as a result of multiple factors, including oxidative stress, damaged DNA, tumour suppressor activation and chronically dysfunctional mitochondria [mai-tow-KON-dree-uh] – the ‘power houses’ of cells.

Dysfunctional mitochondria are a characteristic feature of senescent cells, which when compared with non-senescent cells, exhibit decreased NAD+/NADH ratio, increased production of reactive oxygen species and lower levels of adenosine triphosphate. NAD+/NADH ratio as well as adenosine triphosphate are important molecules for the cell as they can reflect the energetic state of the cell. Alterations in those molecules vastly affect the ability of a cell to proliferate and divide thus lack of NAD and adenosine triphosphate will oppose the transformation process.

Cells destined to become cancerous often have to overcome senescence before they get transformed. Sebastian Igelmann at the University of Montreal, Canada, and his colleagues believe that gaining an understanding of how these cells overcome the obstacle of senescence is vital to aid our understanding of the tumorigenic process and ultimately identify therapeutic targets.

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During senescence caused by specific types of genetic failure, a number of specific proteins are degraded and many cell proliferation processes are affected. One of these proteins is STAT3, which transfers messages within the cell and stimulates specific genetic processes. The protein STAT3 is critical to control nuclear transcription (gene expression) and electron transport and oxidative phosphorylation [fos-fo-ruh-LAY-shn] in mitochondria. The processes of electron transfer and oxidative phosphorylation combine in a system that couple redox reactions, which use the sharing of electrons between molecules to ultimately produce adenosine triphosphate as an energy source.

The presence of functional STAT3 is critical for some oncogenic (cancer-related) proteins to induce cellular transformation, which is the change of normal cells to their tumour state. Furthermore, in some tests in which the STAT3gene is removed from normal cells, mitochondrial dysfunction ensues, reactive oxygen species are overproduced and the cells engage premature senescence also called premature age. All these factors confirm the vital role of STAT3 in senescence and malignant transformation.

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The team has worked to identify the key pathways by which mitochondrial dysfunction may be overcome, consequently enabling potential tumour cells to overcome senescence and proliferate.

They found that when STAT3 is depleted from cells, the cells become senescent and mitochondrial dysfunction is apparent. Structurally, the mitochondria appear to be substantially different to untreated cells of the same type. Furthermore, the team noted that these circumstances resulted in a reduction of the ratio of NAD+/NADH, which is a specialist pairing of redox molecules, vital for electron exchange in metabolism.

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STAT3-depleted cells can be rescued from senescence by the addition of the compounds that normalise the NAD+/NADH balance and thus the redox potential of the cell. Those molecules include pyruvate or duroquinone and the addition of those to the culture medium results in NAD+ regeneration from NADH. The researchers further demonstrated the importance of NAD+/NADH balance by overriding senescence in the STAT3-depleted cells using an enzyme (NADH-oxidase) isolated from a bacterial source.

Thus, they concluded that failing to re-oxidise NADH to produce NAD+ is involved in the induction of cellular senescence.

Having shown that failing to re-oxidise NADH to produce NAD+ induces and maintains cellular senescence and thus opposes the transformation process, the team assessed genetic alterations (mutations) in non-transformed (normal cells) and transformed cells (cancerous cells) that could occur without the artificial addition of metabolites and still oxidise NADH into NAD+.

The team focused on one of the most commonly mutated tumour suppressors, namely p53. They identified that p53 and other tumour suppressors can repress a metabolic cycle that allows regeneration of the NAD+.

They identified this cycle by inactivating tumour suppressors pathways through molecular manipulation, and found that in STAT3-depleted cells, neither growth arrest nor senescence occurred in response to pathway disruption. Furthermore, inactivation of these tumour suppressor pathways also led to the recovery of the NAD+/NADH ratio in the STAT3-depleted cells. Thus, the recovered NAD+/NADH ratio in these cells leads to an appropriate level of energy production and redox potential thus enabling the cells to bypass senescence.

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In further work, Dr Ferbeyre’s group teamed up with the laboratory of Dr Ivan Topisirovic and his students Oro Uchenunu and David Papadopoli, all experts in metabolic isotope tracing, to gain more detailed information on how this novel metabolic cycle is able to regenerate NAD+.

Metabolic tracing takes advantage of a marked carbon isotope to follow the metabolic activities of the cell. Interrogating these pathways showed that the loss of one tumour suppressor path leads to an increase in some important components of metabolism namely pyruvate carboxylase (PC). PC is a central enzyme in metabolism, generating an energy rich metabolite that can be used in downstream metabolic reactions.

In mammals, PC is an enzyme located in the mitochondria. However, in viral infections, for example, PC can relocate to the cytosol, which is the fluid interior of the cell. Consequently, the team proposes that PC in cells that lose tumour suppressor pathways (such as the p53 pathway), a fraction of PC can accumulate in the cytosol. PC in the cytosol can then supply important intermediate compounds for NAD+ regeneration, which occurs via another enzyme found in the cytosol malate dehydrogenase 1 (MDH1).

Finally, their metabolic tracing analysis revealed that Malate, the product of the MDH1 reaction, is found to increase in cells that bypass senescence. The team proposes that this process operates as a closed loop, and that the cytosolic enzyme malic enzyme 1 (ME1) is involved by converting malate back to pyruvate, ultimately regenerating NAD+ and providing NADPH. As mentioned before, both NAD and NADPH are important for the redox homeostasis of the cell.

In short, the novel metabolic cycle is catalysed by MDH1, cytosolic PC and ME1 and regenerates NAD+ to overcome cellular senescence.

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While PC is more abundant in the mitochondria, the researchers have identified a full-length enzyme, and a lower molecular weight form, in the cytosol of a STAT3-depleted prostate cancer cell line. They also note that a cell model of STAT3-depleted cells exhibits localised cytosolic bodies containing PC, MDH1 and ME1.

The colocalisation of the three enzymes indicates that they may interact to form a complex defined as a hydride transfer complex (HTC), a theory that is supported by the co-immunoprecipitation of the three enzymes. In addition, endogenous occurrence of HTC in cells was confirmed by the presence of high molecular weight, heavier than each of the individual components alone, observed during analysis containing the three enzymes.

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To confirm the importance of ME1, MDH1 and cytosolic PC in the generation of NAD+ and NADPH, Mr Igelmann and his colleagues then assessed the ability of the HTC enzymes to override senescence similarly to how was observed by the inactivation of tumour suppressor pathways. The reasoning for this is that a tumour suppressor pathway can be inactivated by directly interfering with the effectors or upstream mechanisms. They hypothesised that ME1, MDH1 and cytosolic PC could be an upstream mechanism that could lead to the inactivation of tumour suppressor pathways. Concordantly, they found that co-expression of the three enzymes resulted in a return to control levels of cellular proliferation. Cells treated in this way also showed reduced expression of the markers of tumour suppression, and reduced DNA damage markers, in combination with a corresponding increase in markers of cell division and proliferation.

Most importantly, these cells exhibited a return to control levels of the NAD+/NADH ratio, and increased cellular NADPH and lower levels of reactive oxygen species.

Overall, it appears that the enzymes of the HTC induce metabolic reprogramming of STAT3-depleted cells, restoring NAD+ levels and increasing NADPH which corresponds to the suppression of senescence. Further investigation led the team to demonstrate that HTC overexpression results in metabolic changes that are equivalent in size to that observed in cells that do not produce the tumour suppressor markers.

Overall, a key finding arising from this work is that HTC enzymes prevent senescence and contribute to malignant transformation, that is the initiation of cancers. The observation that HTC expression in combination with activation of oncogenes allows for tumour formation in a mouse model is a remarkable observation as it points to the possibility of metabolic adaptations that drive tumorigenesis.

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The researchers have assessed several cancer cell lines, human cancers and normal human cells, showing that the HTC enzymes are highly expressed in several cancers, including prostate, metastatic breast cancer, non-small cell lung cancer, glioblastoma, renal tumours and gallbladder tumours, and that, over time, HTC can rescue some cells from senescence.

These findings may indicate that HTC not only operates in malignant transformations that are driven by specific genes, but that it also plays an adaptive role in normal human cells, by increasing the NAD+/NADH ratio and delaying senescence. The research team suggest that HTC may additionally function in cases where adaptation to mitochondrial dysfunction is needed, such as during oxygen deprivation to prevent cytotoxicity as a consequence of increased levels of reactive oxygen species.

There is a body of evidence confirming the associations between individual and multiple components of the HTC, cancer and prognosis. On the basis of their significant work, Mr Igelmann and his colleagues now present the HTC as an important target in the development of much-needed therapies for cancer.

This SciPod is a summary of the paper ‘[A hydride transfer complex reprograms NAD metabolism and bypass senescence](https://linkinghub.elsevier.com/retrieve/pii/S1097-2765%2821%2900695-X)’, published in the journal Molecular Cell, <https://doi.org/10.1016/j.molcel.2021.08.028>

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