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STAT3 and metabolism: how many ways to use a single molecule?

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Signal transducer and activator of transcription (STAT) 3 has been considered a potential anti-cancer target since its first description as an oncogene in 1999[1]. Since then, two STAT3 inhibitors have been brought to clinical trial for the treatment of solid tumors. However, the past 14 years of intense basic research have uncovered novel STAT3 functions that could affect the outcome of the designed therapeutic approach, or could help designing function-specific inhibitors.

STAT3 was originally termed acute phase response factor, for its ability to mediate the increased expression of acute phase proteins in liver cells in response to inflammation[2]. Thereafter, many studies have demonstrated constitutive tyrosine phosphorylation (Y-P) of STAT3, mediating dimerization, nuclear localization and transcriptional activity and correlating with increased expression of its target genes, in a variety of human tumors of both solid and liquid origin[3]. Persistent STAT3 activation mainly occurs downstream of either continuous stimulation by cytokines and growth factors, or constitutive activity/expression of pro-oncogenic tyrosine kinase molecules such as for example cSrc, and only marginally via activating genetic mutations[4]. Indeed, STAT3 Y-P and transcriptional activity are required for tumor transformation downstream of Src and several other oncogenes[5], and interfering with STAT3 leads to growth arrest and apoptotic cell death in the vast majority of tumor cells displaying constitutive activity of this factor[6, 7]. These observations have prompted the development of a number of approaches to inhibit STAT3 transcriptional activity with the goal of treating Y-P STAT3 positive cancers.

However, STAT3 has also been associated to anti-oncogenic functions in thyroid tumors and in late stage APC^{Min} intestinal tumors[8, 9] highlighting the complexity of its functions.

The heterogeneity of STAT3 gene expression signature, in both tumors and tissues of different origin, suggests that a core function for STAT3 in tumors has not been identified yet. On this regard, important connections between STAT3 activities and cell metabolism have recently emerged. First, constitutively active Y-P STAT3 was shown to support a glycolysis-like state through the transcriptional induction of the hypoxia-responsive factor HIF-1α and the down-regulation of mitochondrial activity[10]. Further implications with the regulation of cell metabolism were suggested by the observation that STAT3 can be chronically activated via Y-P by the HIF-1-induced pyruvate kinase M2 isoform (PKM2), starting a positive feedback loop to support cell proliferation and survival[11]. This switch towards aerobic glycolysis may be required for rapid proliferation of cancer cells as well as to drive their plasticity to adapt and survive in environments of different oxygen concentrations, thus partially explaining the addiction for STAT3 shown by so many biologically different tumors[10].

STAT3 encounters other post-translational modifications such as phosphorylation on Serine residue 727 (S-P), which also appears to be involved in the control of metabolic processes. Indeed, S-P STAT3 localizes into the mitochondria, where it preserves oxidative phosphorylation and opposes the opening of the mitochondrial permeability transition pore, thus inhibiting apoptosis[12, 13]. This function was shown to be required for tumor transformation mediated by oncogenic RAS, paradoxically favoring both aerobic glycolysis and ETC activity[14]. STAT3 appears therefore to function as a hub to integrate different oncogenic signals, via both mitochondrial and nuclear activities[15] (Fig. 1). An outstanding question remains how STAT3 would exert its activity within the mitochondrion, since its very low abundance in this organelle challenges the proposed mechanism based on the interaction with specific electron transport complexes of the mitochondrial inner membrane[16].

STAT3 chemical or genetic inhibition accelerates the autophagic flux due to an inhibitory interaction of the STAT3 SH2 domain with the cytoplasmic PKR kinase[17]. Interfering with either the STAT3 cytoplasmic localization, through constitutive or acute Y-P activation, or with its SH2 domain, using specific drugs such as STATTIC or S3I, may in principle increase the pool of free PKR and basal autophagy (Fig. 1). This relatively straightforward mechanism, however, fails to explain why also inhibitors that indirectly prevent STAT3 phosphorylation, such as the Jak kinase inhibitor WP1066, were also able to increase basal autophagy in the absence of constitutively active STAT3 Y-P, suggesting more complicated interplays between the different forms of STAT3.

The novel nuclear, mitochondrial and cytoplasmic functions of STAT3 in the regulation of cellular metabolism are clearly finely tuned in physiological conditions. This balance is likely disrupted in different pathologies, making the dissection of the single activities and the expected outcome of drug treatments more complex.

The two inhibitors under clinical trial are both based on interfering with STAT3 transcriptional activity. The first drug is a decoy oligonucleotide recently assessed in phase 0 trials for the treatment of head and neck cancers[18]. The second drug (OPB-31121) is a small molecule currently in Phase 1 for advanced solid tumors, which down-regulates the expression of the upstream kinase JAK2 and of the signaling receptor gp130, thus indirectly decreasing the rate of STAT3 Y-P[19].

We and others have shown that the chronic transcriptional activity of STAT3 is able to act as a first hit in the process of malignant transformation[20]. Moreover, the level of circulating IL-6, one of the best characterized STAT3-activating factors, increases with age, thus supporting a possible age-dependent chronic STAT3 tyrosine-activation[21]. Since aging is the single factor with the highest impact on cancer initiation, interfering with STAT3 transcriptional activity may be useful as a prophylactic therapy to reduce the rate of cellular

transformation. Systemic prophylactic STAT3 blockade by means of specific inhibitors would presumably be too toxic, due to continued inhibition of its physiological functions, but effective results might be achieved by chronic anti-inflammatory treatments.

Once the tumor is already established, however, inhibiting STAT3 transcriptional activity will eventually increase the pool of monomeric protein in the cancer cells. We have shown that chemically interfering with the constitutively activated form of STAT3 leads to a 'counter-switch' to oxidative phosphorylation[10]. On one side, this effect will initially slow down proliferation and adaptation to a hypoxic environment. On the other side, the increased abundance of monomeric STAT3 may increase its availability for mitochondrial localization, which might eventually lead to enhanced ATP production and glycolysis rate, as previously described[12]. On the other hand, constitutively activated STAT3 might indirectly favor autophagy simply by increasing the pool of active PKR in the cytoplasm; accordingly, many STAT3 inhibitors, including those under clinical trial like the decoy and OPB-31121, lead to an increased pool of cytoplasmic STAT3 able to interact with PKR, and should therefore decrease autophagy, while inhibitors blocking the SH2 domain should enhance it. As autophagy can have both beneficial and pathogenic effects in cancer[22], the end results of such treatments may vary according to the tumor type and stage. STAT3 contrasting functions in tumorigenesis, where this factor can sometimes play oncosuppressive functions, may very well relate to different metabolic needs of specific tumors.

Despite the concept of STAT3 as an oncogene gaining strength during these years, the new twists discussed here suggest that its novel metabolic functions, both transcriptional-dependent and –independent, need to be kept in strong consideration for the development of new therapeutic strategies. First, combinatorial therapies based on inhibiting STAT3 transcriptional activity and interfering with cell metabolism might be redundant; second, therapies based on inhibiting STAT3 transcriptional activity might have different and opposite

outcomes depending on the oncogenic signals activated; third, since cancer cells might rely on STAT3 also independently from its dimerization, its tyrosine phosphorylation might not be a suitable diagnostic for STAT3-dependent cancer.

These considerations underline the need to deepen our knowledge of the role played by the different STAT3 forms (Y-P versus S-P, nuclear versus cytoplasmic or mitochondrial, dimeric versus monomeric) and their relative distribution in different kinds of tumors and downstream of different activated oncogenic pathways. In turn this new knowledge will help defining specific effective inhibition strategies as well as establishing guidelines to assess treatment outcome.

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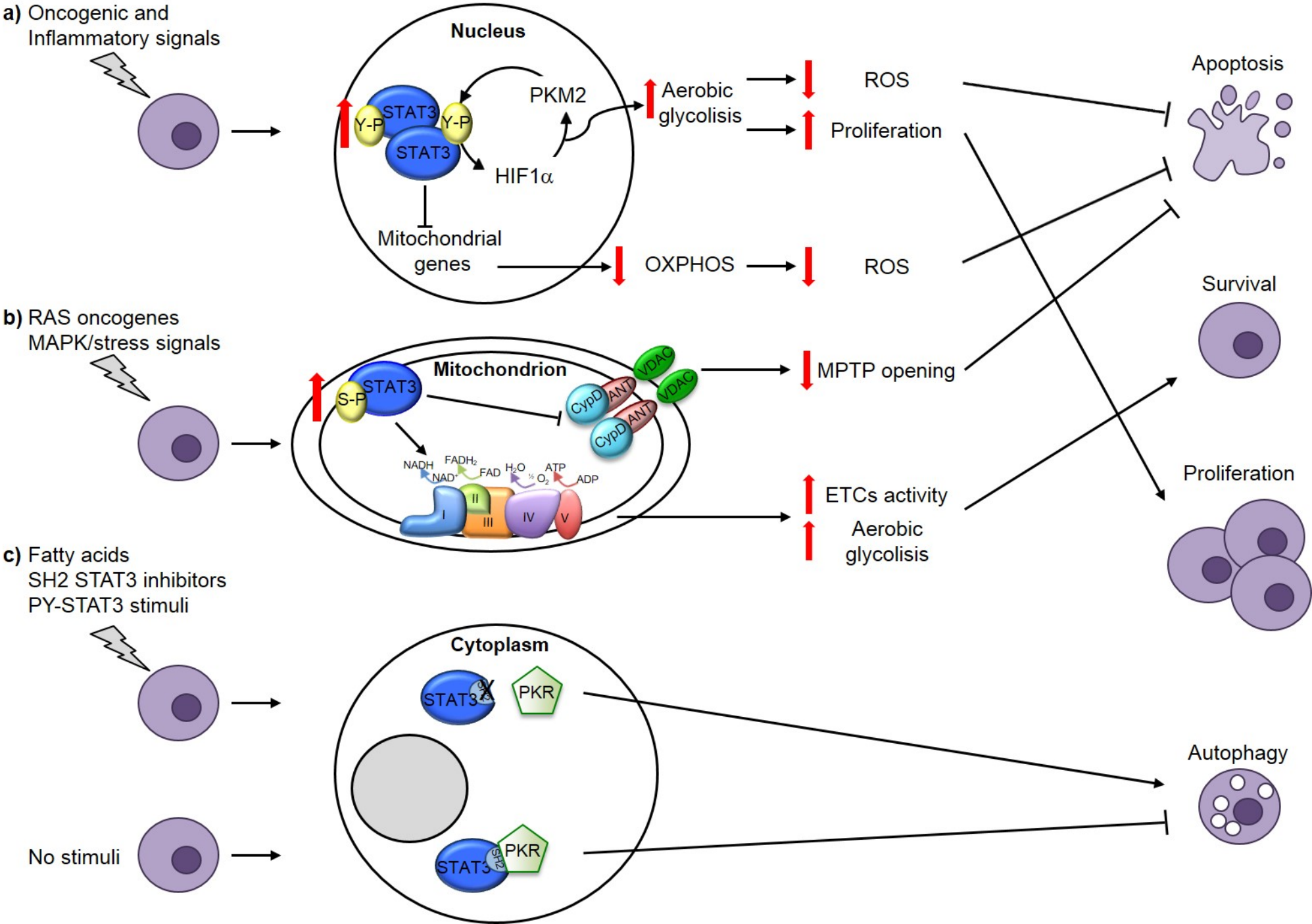
Figure Legend

Multiple metabolic roles of STAT3 according to stimulus, post-transcriptional modification and subcellular localization. In both normal and tumor cells STAT3 can be found in the mitochondrial matrix (mSTAT3), in the nucleus (nSTAT3) and in the cytoplasm (cSTAT3), depending on specific post-transcriptional modifications (i.e. Y-P, S-P or none) that are triggered by different upstream stimuli. **(a)** P-S mSTAT3, induced among others by RAS oncogenic signals and the activation of MAP kinases, can improve cell survival by enhancing both oxidative phosphorylation and aerobic glycolysis, and protects cells from apoptosis by inhibiting the opening of the mitochondrial permeability transition pore (MPTP) via its interactions with the pore component Cyclophylin D (CypD). **(b)** In contrast, many oncogenic signals including inflammatory cytokines, growth factors and oncogenes mainly induce P-Y nSTAT3, which mediates the upregulation of the oxygen sensor Hif-1α thus leading to increased glycolysis and metabolic advantages for proliferating cells. Moreover, STAT3 can be chronically activated by the HIF-1-induced PKM2, thus initiating a positive feedback loop to support cell proliferation and protection from apoptosis and senescence. On the other hand, nSTAT3 decreases mitochondrial activity in an Hif-1α-independent manner by down-regulating a significant number of mRNAs encoding for protein components of the ETC complexes, thus leading to reduce mitochondrial respiration, ROS production and apoptosis. **(c)** Finally, cSTAT3 has been recently shown to inhibit autophagy by interacting via its SH2 domain with the cytoplasmic Protein Kinase R (PKR).

Adenine nucleotide translocator (ANT); Voltage-dependent anion channel (VDAC).


a) Oncogenic and Inflammatory signals
Nucleus
STAT3
Y-P
Y-P
STAT3
PKM2
HIF1α
Mitochondrial genes
Aerobic glycolisis
ROS
Proliferation
OXPHOS
ROS
Apoptosis
Survival
b) RAS oncogenes MAPK/stress signals
Mitochondrion
STAT3
S-P
VDAC
VDAC
CypD
ANT
CypD
ANT
MPTP opening
NADH
NAD+
FADH2
FAD
H2O
½ O2
ATP
ADP
I
II
III
IV
V
ETCs activity
Aerobic glycolisis
Proliferation
c) Fatty acids SH2 STAT3 inhibitors PY-STAT3 stimuli
Cytoplasm
STAT3
PKR
No stimuli
STAT3
PKR
Autophagy