GSK-3 and mitochondria in cancer cells

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INTRODUCTION

GSK-3 is a multifunctional kinase that is located in the cytosol, nucleus, and mitochondria of all cell types, and it is involved in the pathogenesis of a variety of diseases. In cancer, GSK-3 modulates the response of the cell death machinery to stress stimuli, including chemotherapeutics. Mitochondria are at the heart of the integration between survival and noxious signals; therefore, modulation of the mitochondrial functions carried out by GSK-3 is profoundly involved in the apoptosis escape capabilities that hallmark neoplasms. This review briefly covers the mechanistic interactions among oncogenic kinase pathways, GSK-3 activity and subsequent modulation of mitochondrial functions that shape the pro-survival phenotype of cancer cells, such as control of redox homeostasis and inhibition of the mitochondrial permeability transition pore.

Keywords: GSK-3, mitochondria, ROS, chemotherapeutics, PTP, cancer, apoptosis
FIGURE 1 | GSK-3 modulates mitochondrial PTP opening downstream to kinase signaling activated during neoplastic transformation. The active form of GSK-3 phosphorylates both Bax and VDAC. Bax is activated and migrates to the outer mitochondrial membrane (OMM), where it oligomerizes and induces membrane permeability; phosphorylated VDAC becomes a consensus site for Bax which displaces HK II from the same binding site. The mitochondrial fraction of GSK-3 can facilitate PTP opening by CyP-D phosphorylation. Ser phosphorylation of both cytoplasmic and mitochondrial GSK-3 by several kinases, such as AKT and ERK, critically contributes to defuse the mitochondrial apoptotic machinery downstream to ligand- or oncogenically activated receptor tyrosine kinases. GSK-3 inhibitory phosphorylation in enhanced by regulatory loops that involve ERK and p38 MAPK targeting other Ser and Thr residues on GSK-3. PTP inhibition is elicited both by dephosphorylated VDAC, which has high affinity for Thr 5 and competitively displaces Bax from the OMM, and by active mitochondrial ERK, which inhibits mGSK-3 by Ser phosphorylation, in turn blocking CyP-D phosphorylation and PTP induction. Contextually, the absence of GSK-3-dependent Bax activation inhibits Bax relocation in the OMM.

2007). In this complex, co-localization of GSK-3 with a specific subset of binding proteins favors selective protein–protein interactions, leading to CK1-dependent priming of β-catenin, which is subsequently phosphorylated by GSK-3 and tagged for proteasomal degradation, thus blocking its transcription activity that favors cell proliferation (Wu and Pan, 2010).

GSK-3 AND Bcl-2 FAMILY PROTEINS
GSK-3 contributes to the anti-apoptotic phenotype of cancer cells by controlling the mitochondrial localization and the activation status of a number of proteins of the cell death machinery, shaping the ability of cell death escape that hallmarks malignancies. Numerous proteins relevant to cell death [i.e., Mcl-1, Bcl-2, Bax, Nona, voltage-dependent anion channel (VDAC), and adenosine nucleotide transporter (ANT)] are board of GSK-3 and are located or can translocate in mitochondria. As a general rule, intrinsic apoptosis elicited by a variety of stress conditions that can be encountered by a malignant cell, such as withdrawal of growth factors, chemotherapeutics, or oxidant stress, is facilitated by active GSK-3 (Beurel and Jope, 2006). Thus, GSK-3 activity potentially counteracts neoplastic transformation. The phosphorylation of targets located on the external surface of mitochondria does not strictly require a mitochondrial localization of GSK-3, but it is plausible to envisage that the enzyme must be associated to outer membrane components. GSK-3 regulates several members of the B-cell lymphoma-2 (Bcl-2) protein family: it
prompts phosphorylation-mediated proteasomal degradation of the anti-apoptotic protein myeloid cell leukemia-2 (Mcl-2) (Mauer et al., 2006; Ding et al., 2007), whose expression correlates with Ser-phosphorylation dependent inactivation of GSK-3 in diverse cancer cell types (Ding et al., 2007b), whereas a decreased phosphorylation of potential GSK-3 target sequences on Bcl-2 itself contributes to its anti-apoptotic activation (Juhászova et al., 2009). GSK-3 inhibition abolishes both the mitochondrial translocation and the conformational activation of the pro-apoptotic protein Bcl-2-associated X (Bax) through direct phosphorylation of a Ser residue on Bax found within a putative GSK-3 phosphorylation motif; and a constitutively active GSK-3 prompts Bax localization to mitochondria (Linsenmair et al., 2004; Ofori et al., 2008; Ge et al., 2012; Ngok-Ngam et al., 2013). In contrast with these observations, it was shown in human colorectal cancer cells that pharmacologic inhibition of GSK-3 elicited p53-dependent conformational activation of Bax, resulting in apoptosis induction (Tan et al., 2005). Moreover, treatment of melanoma cells with the multiple kinase inhibitor sorafenib activates GSK-3, leading to down-modulation of the pro-apoptotic Bcl-2 family member Noxa (Panksa et al., 2008). These findings suggest that, at least in some neoplastic models, GSK-3 inhibition could enhance apoptosis.

**GSK-3 AND HEXOKINASE II**

GSK-3 also regulates tumor cell survival by controlling mitochondrial binding of hexokinase, particularly hexokinase type II (HK II), which is highly expressed on the outer mitochondrial membrane (OMM) of most cancer cells. HK initiates the process of intracellular glucose utilization and it contributes to the Warburg effect, i.e., to the uncoupling between glycolysis enhancement and oxygen availability (Warburg, 1956; Hsu and Sabatini, 2008), supporting cell proliferation in the hypoxic conditions of primary tumor mass accrual. Association of HK II to the OMM is enhanced when GSK-3 is inactivated through phosphorylation by the survival kinase Akt, whose signaling is constitutively enhanced during chronic liver inflammation, induces a ROS-dependent activation of mGSK-3 that causes depletion of mitochondrial DNA in human hepatic cells (Vadrot et al., 2012). mGSK-3 is the point of convergence of several transduction pathways that regulate PTP opening following ischemia/reperfusion in the heart (Juhászova et al., 2004), including the survival kinases Akt and Erk1/2, PKCβ, protein kinase G (PKG) and p70s6K (Hausenloy and Yellon, 2007); notably, effectiveness of ischemic pre- and post-conditioning in preserving cardiomyocyte viability requires mGSK-3 inhibition through Ser phosphorylation, which in turn inhibits the PTP in response to ROS or Ca2+ overload (Juhászova et al., 2009; Miura and Miki, 2009; Miura et al., 2010). Accordingly, a significant increase in active, Ser-dephosphorylated mGSK-3 is observed during ischemia (Miura and Tanno, 2012).

The molecular mechanisms that regulate the mitochondrial pool of GSK-3 and in turn the PTP in cardiomyocytes could be relevant to neoplasms too, strongly contributing to the anti-apoptotic phenotype of tumor cells. Indeed, several data indicate that PTP dysregulation has a role in tumorigenesis, increasing resistance of neoplastic cells to a variety of stressful conditions such as exposure to chemotherapeutics, hypoxia, or detachment from the extracellular matrix (Rasola et al., 2010b). Even if the lack of a molecular characterization of the PTP hampers a thorough characterization of its modulation by GSK-3, it is conceivable that mGSK-3 could contribute to PTP regulation both by acting as a downstream effector of diverse signaling pathways, and by changing mitochondrial
ROS levels, as ROS are well-established PTP inducers (Rasola and Bernardi, 2011). Tumor cells are particularly exposed to the noxious effects of loss of redox homeostasis, as they are endowed with abnormally high ROS levels (Cairns et al., 2011). Thus, by modulating the PTP mGSK-3 could crucially affect the survival potential of neoplastic cells. Accordingly, it was observed that mGSK-3 activation enhances ROS production and apoptosis following treatment of neurons and of human neuroblastoma cells with complex I inhibitors (King et al., 2008; Petit-Pail et al., 2009). Moreover, we and others have observed that cyclophilin D (CyP-D), a mitochondrial chaperone that regulates the PTP (Rasola and Bernardi, 2007), is directly phosphorylated by GSK-3 on Ser/Thr residues in tumor cell models (Rasola et al., 2010a; Totta et al., 2011) or in cells lacking mitochondrial DNA and characterized by a Warburg-like metabolic phenotype (Masgras et al., 2012). We found that a portion of ERK locates in the mitochondrial matrix, and mitochondrial ERK, which turned out to be constitutively active after v-Ki-Ras dependent transformation or in diverse neoplastic cell types, inhibits mGSK-3 by Ser phosphorylation, thus conferring resistance to death stimuli acting as PTP inducers (Rasola et al., 2010a). Moreover, ERK inhibition increased GSK-3-dependent phosphorylation of CyP-D and sensitization of PTP to opening, thus significantly abolishing tumor cell protection from apoptosis, whereas pharmacological inhibition of GSK-3 protected from PTP opening (Rasola et al., 2010a).

**FIGURE 2** | Molecular mechanisms elicited by the recently synthesized gold-compound AUL12 to specifically induce cell death of tumor cells. AUL12 inhibits the complex I of the mitochondrial respiratory chain thus eliciting an increase in ROS levels. The ROS surge reactivates the oncogenically inhibited GSK-3, mitochondrial GSK-3 phosphorylates CyP-D, unlocking the PTP blocked by oncogene signaling; cytosolic GSK-3 binds to and phosphorylates Bax, leading to its mitochondrial translocation. Mitochondrial Bax prompts mitochondrial permeabilization at least partially by inducing the PTP.

GSK-3, MITOCHONDRIA AND CHEMOTHERAPY

GSK-3 displays a multiplicity of functions in distinct cellular compartments and in a variety of cell types, and it is involved in several disorders. This makes GSK-3 an interesting target for drug discovery, but at the same time considering GSK-3 as a therapeutic target exposes to the risk of undesired side effects, particularly when patients are treated in a chronic mode. During the last decade, a priority has been given to the search for GSK-3 inhibitors, and promising data exist for the treatment of neurological disorders and diabetes (Gould et al., 2006; Gao et al., 2011). In the field of cancer chemotherapy, GSK-3 mainly acts as a tumor suppressor by inhibiting many proto-oncogenic proteins and tumor development. Nonetheless, in neoplasms such as human ovarian, colon, hepatic and pancreatic carcinomas, some studies suggest that GSK-3 may actually exert a pro-neoplastic function, and inactivation of GSK-3 is associated with growth suppression of medullary thyroid cancer cells (Luo, 2009). As a consequence, targeting GSK-3 with drugs that could act as anti-neoplastic agents is an extremely complicated issue. For instance, GSK-3 displays a tumor suppressor activity in mammary tumors, and its activation causes sensitization to chemotherapeutics of breast cancer cells, but in colon cancer, where the enzyme is a tumor promoter, GSK-3 must be inhibited to increases the effect of chemotherapy (Luo, 2009). These paradoxical observations are probably explainable by the pleiotropy of GSK-3 functions, and a better comprehension of...
As an example, in hepatoma cells the effect of chemotherapy can be enhanced by pharmaceutical inhibition of the PI3K signaling pathway, as this reactivates GSK-3 and facilitates mitochondrial translocation of Bax and the ensuing apoptosis (Beurel et al., 2005).

In this conceptual framework, we have recently characterized the mitochondrial effects of the GoldIII-dithiocarbamate complex AUL12, a gold-based chemotherapeutic of new generation designed with the specific aim of improving selectivity, bioavailability, and efficacy of platinum-based compounds, diminishing their toxic side effects (Ronconi and Fregona, 2009). It was observed that AUL12 increases intracellular ROS levels (Saggioro et al., 2007), therefore, our idea was to target the increased ROS levels that characterize tumor cells. As cancer cells are forced to induce anti-oxidant defenses to set a novel homeostatic redox equilibrium, we reasoned that a further increase in ROS levels could overwhelm their residual anti-oxidant capabilities, triggering PTP opening and cell death in a selective way, i.e., without a major damage to non-transformed cells. We observed (Chiara et al., 2012) that AUL12 elicits a rapid burst of mitochondrial superoxide levels following inhibition of the RC complex I, which causes GSK-3\(\alpha\)/\(\beta\) phosphorylation and activation. The mitochondrial fraction of GSK-3\(\beta\) phosphatase CyP-D, which in turn facilitates PTP opening, whereas the cytosolic GSK-3\(\beta\) interacts with Bax and prompts its mitochondrial translocation, where it contributes to PTP induction and tumor cell death (Figure 2).

Notably, AUL12 was much less toxic on non-transformed cells and after in vivo administration (Marranzino et al., 2011). These findings provide evidence that targeting specific signaling pathways maintained by mitochondria in tumor cells allow to shut crucial mechanisms that shield neoplasms from the toxicity of many anti-neoplastic strategies, and pave the way for the design of a new family of chemotherapeutic compounds that sensitize cancer cells to chemotherapy.

**REFERENCES**

Rahman, L. M., Tian, P. Y., Lin, H. Y., Jiang, Y. P., and Lin, R. Z. (2011). Dual regulation of glycogen synthase kinase-beta by the alpha1-adrenergic receptor. J. Biol. Chem. 286, 9405–9410.

Beurel, E., and Jope, R. S. (2006). The paradoxical pro- and anti-apoptotic actions of GSK3\(\beta\) in the intracellular and extracellular apoptotic signaling pathways. Prog. Neurobiol. 79, 173–189.

Beurel, E., Kornprobst, M., Blivet-Van Egmond, M. J., Cadoret, A., Caprane, L., and Dodson-Mooreton, C. (2008). GSK-3\(\beta\) and Akt facilitate mitochondrial translocation of Bax in hepatoma cells. J. Biol. Chem. 283, 1294802 sensitizes hepatoma cells to chemotherapy-induced apoptosis. J. Biol. Chem. 283, 2175–2222.

Braie, M. J., Bourbonnais, F. J., and Salifu, A. B. (1998). The activation of glycogen synthase by insulin stimulation of glycogen synthase kinase-3beta in human hepatoma cells. J. Cell Biochem. 57, 14063–14066.

Chiara, F., Gambalunga, A., Sciacovelli, T. W. (2011). Regulation of cancer cell metabolism. Nat Rev Cancer 11, 85–93.

Chiara, F., Candillaro, D., Marin, O., Petronilli, V., Brusilow, W. S., and Jope, R. S. (2008). Nitric oxide dissociation from mitochondria triggers apoptosis through the permeability transition pore independent of voltage-dependent anion channels. PLos ONE 3, e2882. doi:10.1371/journal.pone.0002882

Chiara, F., Gambalunga, A., Sciencetti, M., Nicolli, A., Brusilow, W. S., and Jope, R. S. (2012). Chemotherapeutic induction of mitochondrial oxidative stress activates GSK-3\(\alpha\)/\(\beta\) and Bax, leading to permeability transition pore opening and tumor cell death. Cell Death Dis. 3, e444.

Cola, A., Frasca, S., and Cohen, P. (2004). Further evidence that the tyrosine phosphorylation of glycogen synthase kinase-3 (GSK-3) in mammalian cells is an autophosphorylation event. Biochim. Biophys. Acta 1691, 249–255.

Ding, Q., He, X., Hu, J., Mia, X., Wu, R., Li, H., et al. (2008). Upregulation of MnSOD in a betaTC10A mutant glycogen synthase kinase\(\beta\)-mediated tumor suppression and chemoresistance. J. Biol. Chem. 283, 12726–12736.

Ding, Q., He, X., Xia, W., Hu, J. M., Chen, C. T., Li, L. Y., et al. (2007). Myrckyl cell keratin 1 invariably correlates with glycogen synthase kinase-3beta activity and associates with poor prognosis in human breast cancer. Cancer Res. 67, 5546–5551.

Fang, X., Yu, S., Tan, P. L., Lu, Y., Woodgett, J. R., and Millik, G. B. (2002). Convergence of multiple signaling cascades at glycogen synthase kinase\(\beta\) and associates with poor prognosis in human breast cancer. Cancer Res. 67, 5546–5551.

Fang, X., Yu, S., Tan, P. L., Lu, Y., Woodgett, J. R., and Millik, G. B. (2002). Convergence of multiple signaling cascades at glycogen synthase kinase\(\beta\) and associates with poor prognosis in human breast cancer. Cancer Res. 67, 5546–5551.

Fang, X., Yu, S., Tan, P. L., Lu, Y., Woodgett, J. R., and Millik, G. B. (2002). Convergence of multiple signaling cascades at glycogen synthase kinase\(\beta\) and associates with poor prognosis in human breast cancer. Cancer Res. 67, 5546–5551.

Fang, X., Yu, S., Tan, P. L., Lu, Y., Woodgett, J. R., and Millik, G. B. (2002). Convergence of multiple signaling cascades at glycogen synthase kinase\(\beta\) and associates with poor prognosis in human breast cancer. Cancer Res. 67, 5546–5551.

Fang, X., Yu, S., Tan, P. L., Lu, Y., Woodgett, J. R., and Millik, G. B. (2002). Convergence of multiple signaling cascades at glycogen synthase kinase\(\beta\) and associates with poor prognosis in human breast cancer. Cancer Res. 67, 5546–5551.
Majewski, N., Nogueira, V., Robey, R., B., and Hay, N. (2004). Akt inhibits apoptotic downstream of Rho signaling via a glucose-dependent mechanism involving mitochondrial hexokinase. Mol Cell Biol. 24, 14314–14320.

Majewska, N., Neugent, V., Robey, R., B., and Hay, N. (2004). Akt inhibits apoptotic downstream of Rho signaling via a glucose-dependent mechanism involving mitochondrial hexokinase. Mol Cell Biol. 24, 730–740.

Mariano, C., Ronconi, L., Chiara, F., and Ronconi, L. (2011). Gold(III)-dithiocarbamate antimycotics act on cytoplasmic and histopathological studies in rodents. Int. J. Cancer 129, 487–493.

Mannino, L., Rassouli, A., and Bernardi, P. (2012). Induction of the permeability transition pores in cells depleted of mitochondrial DNA. Biochim. Biophys. Acta 1823, 2486–2496.

Marinapoulou, S. K., Kay, H., and Pedron, E. L. (2006). Hexokinase II: cancer’s double-edged sword acting as both facilitator and gatekeeper of metabolic reprogramming in cancer. Oncogene 25, 4777–4789.

Maurice, J. C., Ciment, C., Goga, A. S., Dourdel, E., and Green, D. R. (2004). Glycogen synthase kinase-3 regulates mitochondrial outer membrane permeabilization and apoptosis by destabilization of permeability transition complex. Mol. Cell 21, 749–756.

Mochida, M., Giraldo, J. I., and Wanders, F. G. (2011). Modulation of GSK-3 as a therapeutic strategy on glucose metabolism. Front. Mol. Neurosci. 4:24. doi: 10.3389/fnmol.2011.00024

Munoz, T., and Tanno, M. (2010). Mitochondrial kinase signaling pathways in myocardial protection of cytosolic and mitochondrial GSK-3beta. Biochem. J. 433, 563–570.

Murphy, M. P. (2008). How mitochondria produce reactive oxygen species. Bioessays 30, 141–149.

Nir, M., Tanno, M., and Sato, T. (2012). Mitochondrial kinase signaling pathways in myocardial protection of intact cells via serine 9 phosphorylation. J. Biol. Chem. 287, 750–752.

Pahl, D. J., Cho, D. C., Jenkins, M. B., and Mier, J. W. (2008). GSK-3β inhibition enhances serine/threonine phosphorylation in melanoma cell lines. J. Biol. Chem. 283, 750–752.

Papandreou, A., Bisos, F., Carnevali, G., and Chafer, Z. (2008). Involvement of cytosolic and mitochondrial GSK-3beta in mitochondrial dysfunction and cell death of MPTP/MPP-treated neurons. PLoS ONE 3, e2246. doi: 10.1371/journal.pone.0002246

Peled, I. (2007). The many ways of Wnt in cancer. Curr. Opin. Genet. Dev. 17, 45–51.

Rassouli, A., and Bernardi, P. (2007). The mitochondrial permeability transition pores and its involvement in cell death and in disease pathogenesis. Apoptosis 12, 835–835.

Rassouli, A., and Bernardi, P. (2011). Mitochondrial permeability transition in Ca(2+)-dependent apoptosis and necrosis. Cell Calcium 50, 222–233.

Rassouli, A., Sciancoelli, M., Chiara, F., Pantic, R., Bradlow, W. S., and Bernardi, P. (2010). Activation of mitochondrial GSK-3 promotes cancer cells from death through inhibition of the permeability transition pore. Proc. Natl. Acad. Sci. U.S.A. 107, 725–731.

Rassouli, A., Sciancoelli, M., Pantic, R., and Bernardi, P. (2010). Signal transduction to the permeability transition pore. FEBS Lett. 584, 1999–1996.

Robey, R. B., and Hay, N. (2006). Mitochondrial hexokinase, novel modulators of the antipotptic effects of growth factors and Akt. Oncogene 25, 4685–4696.

Ronconi, L., and Fregona, D. (2009). The Mitos touch in cancer chemotherapy: from platinum to gold-dithiocarbamate complexes. J. Inherit. Metab. Dis. 4:217. doi: 10.1007/s10545-009-9260-1

Sadeghi, D., Rigobaldo, M. P., Palousi, L., Föls, A., Mozyczk, S. A., Preuss, S., et al. (2007). Gold(III)-dithiocarbamate complexes induce cancer cell death triggered by tyrosine kinase receptor inhibition and activation of ERK pathway. Chem. Biol. 14, 1126–1139.

Saito, Y., Yando, A., and Cohen, P. (1994). The mechanism by which epidermal growth factor inhibits glycogen synthase kinase 3 in A431 cells. Biochem. Biophys. Res. Commun. 193, 27–31.

Shaw, M., and Cohen, P. (1999). Role of protein kinase B and the MAP kinase cascade in mediating the EGFr-dependent inhibition of glycogen synthase kinase 3 in Swiss 3T3 cells. J. Biol. Chem. 274, 120–124.

Stambolic, V., and Woodgett, J. R. (1994). Mitogen inactivation of glycogen synthase kinase-3 beta in intact cells via serine 9 phosphorylation. Biochem. J. 303(Pt 3), 703–704.

Tan, J., Zhang, L., Living, H. S., Iyer, N. G., Liu, E. T., and Yu, Q. (2015). Pharmacologic modulation of glycogen synthase kinase-3-beta in preventing cell death and protecting against necrosis. Oncogene. 34, 1099–1107.

Tanno, M., Yano, T., Naitoh, K., Iwase, T., Vo, T. A., et al. (2004). Glycogen synthase kinase-3beta and p53 in response to oxidative stress and neuronal cell death of rats. FEBS Lett. 550–554.

Varela, A. T., Simoes, A. M., Teodoro, R., Abreu, A., Perego, C., Dossena, M., Ragni, M., et al. (2011). Glycogen synthase kinase-3 inhibition reduces ischemic reperfusion-induced myocardial injury via stabilization of cyclophilin D. J. Physiol. 590, 4049–4060.