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Glycogen synthase kinase – 3 and cancer - an overview

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ABSTRACT

Defective apoptosis (programmed cell death) represents a major causative factor in the development and progression of cancer. Glycogen Synthase Kinase-3 (GSK-3) has the perplexing capacity to either increase or decrease the apoptotic threshold. These apparently paradoxical effects now are known to be due to GSK-3 which oppositely regulating the two major apoptotic signaling pathways. GSK-3 promotes cell death caused by the mitochondrial intrinsic apoptotic pathway, but inhibits the death receptor-mediated extrinsic apoptotic signaling pathway. Consequently, inhibitors of GSK-3 provide protection from intrinsic apoptosis signaling but potentiate extrinsic apoptosis signaling. So, ability of GSK-3 to oppositely influence two types of apoptotic signaling have thrown a light on important regulatory mechanisms in apoptosis and henceforth designing the rational use of GSK-3 inhibitors for therapeutic interventions in cancer.

Introduction

Cancer is a highly heterogenous disease, arising from multiple tissue types and displaying great genetic diversity. However, recent insight has encouraged some to suggest that the underlying etiology and progression of the disease can be reduced to two lesions, mutations that give rise to excessive proliferation and compensatory disruption of survival signaling pathways that ensures the persistence of hyperproliferative cells. [1] This theory forgoes the fundamental link between neoplasia and apoptosis, as exemplified by the ability of oncogenes including Myc and tumor suppressors such as p53 to actively engage apoptosis. The ability of Myc to drive apoptosis in addition to providing a potent proliferative signal is interpreted as a failsafe mechanism to offset its oncogenic capacity. [2] A variety of intracellular signaling events are activated in response to apoptotic stimuli, their precise role in regulating the downstream core apoptotic machinery. Cao et al. reports that GSK-3β plays an important role in positively regulating the proliferation of human cancer cells. [3] The two most common apoptotic pathways are the 'intrinsic' pathway in which cellular stresses disrupt mitochondrial integrity and 'extrinsic' pathway that is initiated by stimulation of 'death receptors' in the plasma membrane. [4]

GSK-3 is a serine/threonine kinase expressed as two similar isoforms - α and β - that are abundant in most tissues. GSK- 3α has a mass of 51 kDa, whereas GSK-3β is a protein of 47 kDa. The difference in size is due to glycine-rich extension at the N-terminus of GSK-3α. (Fig.1)

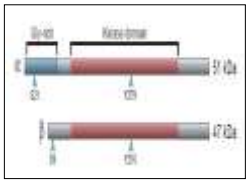

Fig. 1 Structure of Glycogen Synthase Kinase - 3

GSK-3 has several unique features compared with other kinases like

(1) GSK-3 is basally active and extracellular stimulation inhibits GSK-3 kinase activity, while other kinases are activated by ligand.

(2) Phosphorylation at Ser-21 for GSK-3α and Ser-9 for GSK-3β inhibits GSK-3 kinase, while most other kinases are activated by phosphorylation.

(3) GSK-3 requires 'primed' phosphorylation of its substrates by other kinases before GSK-3 can phosphorylate them, except for some cases including β-catenin and Axin.

(4) GSK-3 mainly plays a role as a negative regulator in signaling processes, except in the NF-kB signaling pathway. [5]

Regulation of GSK-3

Both isoforms of GSK-3 are constitutively active in resting cells, but their actions are tightly controlled by four mechanisms shown in fig. 2.

1)The phosphorylation state of GSK-3 substrates (Primed substrate)

2)Phosphorylation of GSK-3 itself

3)The subcellular localization of GSK-3

4)The formation of protein complexes containing GSK-3

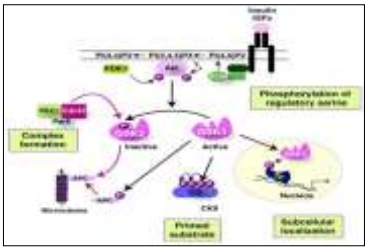

Fig. 2 Regulation of GSK-3 by various mechanisms

1. Primed substrate: GSK-3 contains a consensus sequence of Ser/Thr–X–X–X–Ser–P/Thr–P.

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The first mediated phosphorylation has a preference for targets which have already been phosphorylated or ‘‘primed’’ such that it is 100–1000 folds more efficient on primed substrates. [7] For example, phosphorylation of glycogen synthase by GSK-3 is dependent upon prepriming via Casein Kinase-2 (CK-2) [8] thus factors regulating CK-2 will in effect also be regulating GSK-3.

2. Phosphorylation of GSK-3 itself: The major mechanism for inhibiting the activity of GSK-3 is by serine-phosphorylation, so activity is inhibited when serine-21 of GSK-*3α* or serine-9 of GSK-*3β* is phosphorylated. Conversely, the activity of GSK-3 is optimal when phosphorylation is on tyrosine-279 of GSK-*3α* or tyrosine-216 of GSK-*3β.* Many kinases are capable of phosphorylating the regulatory serines of GSK-*3α/β.* The figure shows this being carried out by Akt (also known as protein kinase B) which itself is activated by phosphorylation on two sites, one mediated by phosphoinositide dependent kinase-1 (PDK1) and the other by an unidentified kinase. Akt activation follows stimulation-induced activation of PI3K and its catalysis of the formation of 3′-phosphoinositides, by receptors for insulin, insulin-like growth factors (IGFs) and other receptor subtypes. When the substrate is prephosphorylated & GSK-3 is active, with the regulatory serine dephosphorylated, two spatial restrictions also contribute to regulate the actions of GSK-3, its subcellular localization and its association with other proteins in regulatory complexes.

3. Subcellular Localization of GSK-3: GSK-3 is considered to be largely a cytosolic enzyme, but it is also associated with or internalized in, subcellular compartments such as the nucleus, mitochondria and growth cones, so dynamic regulation of the subcellular localization of GSK-3 can regulate its access to substrates within subcellular compartments. The example depicted shows GSK-3 transport into the nucleus where it can phosphorylate a variety of substrates including several transcription factors (TF).

4. Complex Formation: In addition to this gross cellular distribution of GSK-3, its distribution in the cell is constrained by its propensity to be associated in protein complexes which provides an important mechanism for regulating its phosphorylation of specific substrates that are colocalized in such complexes. The example shows that a complex of cdc42, Par6, and protein kinase C-*ζ* (PKC*ζ*) binds GSK-3*β* and catalyzes the phosphorylation of serine-9 to inhibit GSK-3*β* to regulate the phosphorylation of APC and thereby control the association of APC with the plus-end of microtubules. Conversely, the enzymatic activity of GSK-3 is enhanced by phosphorylation of tyrosine-216 in GSK-3*β* and tyrosine-279 in GSK-3*α*, but the mechanisms regulating this modification are not well-defined. [9]

GSK-3 and Apoptosis

Apoptosis, a biochemical cascade activates proteases that destroy molecules that are required for cell survival and mediate a program of cell suicide. Carefully regulated cell death by apoptosis is crucial in the development and homeostasis of all multi-cellular organisms. This is emphasized by the prevalence of diseases associated with abnormal apoptosis. For example, deficient apoptosis is a hallmark of cancer and autoimmune diseases, whereas excessive cell death occurs in several neurodegenerative diseases. The two most common apoptotic pathways are the ‘intrinsic’ pathway in which cellular stresses disrupt mitochondrial integrity and the ‘extrinsic’ pathway that is initiated by stimulation of ‘death receptors’ in the plasma membrane. [4] Both apoptotic pathways culminate in the activation of a family of intracellular cysteine proteases called caspases. These are classified as initiator caspases (caspases 8, 9, 10) or effector caspases (caspases 3, 6, 7), [10] which can disrupt entire cells within a few minutes of their activation. [11] GSK-3 gives the paradoxical activity in apoptosis by either strongly inhibiting or strongly promoting apoptotic signaling.

Mitochondrial intrinsic apoptosis pathway

GSK-3 is positioned to be associated with apoptotic events which involve the mitochondria-mediated intrinsic apoptotic pathway as GSK-3 is present within mitochondria. The intrinsic apoptotic signaling cascade can be induced by numerous stimuli that cause cell damage such as DNA damage, oxidative stress, endoplasmic reticulum (ER) stress and many other insults. [12] These conditions those activate intrinsic apoptotic signaling cause the disruption of mitochondria, leading to cell destruction promoted by GSK-3. Cytochrome C released from mitochondria increase the permeability transition pore complex in mitochondria [14] and of the activation of the pro-apoptotic Bcl-2 family members Bax and Bcl-2 interacting mediator of cell death (Bim). [15-17] GSK-3 can directly phosphorylate Bax on Ser-163, which results in the activation of Bax [20] and GSK-3 is required for the stress-induced expression of Bim. [17] Additionally, phosphorylation by GSK-3 enhanced the degradation of the anti-apoptotic Bcl-2 family member MCL-1. [18] GSK-3 also phosphorylates the voltage-dependent anion channel (VDAC) - an abundant outer mitochondrial membrane protein implicated in maintaining the mitochondrial membrane potential. This phosphorylation prevents hexokinase II from associating with VDAC, [23] which may facilitate the mitochondrial association of pro-apoptotic Bcl-2 family proteins to promote apoptosis. [20-22] Thus, one way GSK-3 promotes the intrinsic apoptotic signaling pathway is to expedite signals that contribute to the disruption of mitochondria**.**

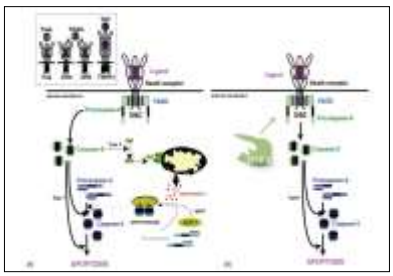

Fig 3. Various pathways leading to apoptosis

A - Mitochondrial intrinsic apoptosis pathway

B - Death receptor-mediated extrinsic apoptosis pathway

Death receptor-mediated extrinsic apoptosis pathway

In the extrinsic apoptotic signaling pathway four major apoptosis-inducing death receptors are activated when Fas ligand (FasL) activates Fas, TRAIL (TNF-Related Apoptosis-Inducing Ligand) activates DR4 or DR5 or TNF activates TNF-R1. The binding of the ligand to the death receptor induces trimerization of the receptor which produces a conformation that recruits FADD and procaspase-8 to the cytoplasmic tail of the receptor, altogether forming a protein complex known as the DISC. [23] Within the DISC, caspase-8 is activated by auto-cleavage. [24] In type I cells, sufficient active caspase-8 is generated to directly activate caspase-3 to carry out the apoptotic program. In type II cells, activation of caspase-8 leads to activation of caspase-3 indirectly through cleavage and activation of Bid, forming tBid, which activates the mitochondrial apoptotic mechanisms that are involved in the intrinsic apoptotic pathway. [24] GSK-3 inhibits the extrinsic

apoptotic pathway by impairing transduction of the signal from activated death receptors to the activation of caspase-8.

Conclusion:

Among the large number of substrates and signaling pathways impacted by GSK-3, Apoptosis regulation is of particular interest because of intriguing dual nature of the regulatory effects of GSK-3. Cancer may be the result of DNA damage due to carcinogens or spontaneously during DNA replication. Inability to correct the DNA damage due to mutated DNA repair genes or absence of functional cell cycle checkpoint genes may give the cell a growth advantage. Increased GSK-3 activity lowers the threshold for activating the intrinsic apoptotic pathway, so pharmacological inhibition of GSK-3 raises the threshold for signals that activate this mechanism of programmed cell death. This reduction of the intrinsic apoptotic threshold by GSK-3 is mediated by the regulatory actions of GSK-3 on proteins involved in causing mitochondrial disruption and the expression levels of apoptotic-regulating proteins. Thus, studies of the role of GSK-3 as a cellular sensor that integrates stimuli that activate apoptosis have revealed its dual functions to promote intrinsic and inhibit extrinsic, apoptotic signaling.

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