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Nuclear importin α and its physiological importance

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Importin α is recognized as a classical nuclear localization signal (cNLS) receptor which mediates nucleocytoplasmic transport. However, it rapidly accumulates in the nucleus in response to cellular stresses, including oxidative stress, causing a blockade of the classical nuclear import pathway. We set out to determine whether importin α performs roles in the nucleus after cellular exposure to stresses and discovered that it can act directly to modulate gene expression. With remarkable selectivity, importin α2 can access the promoter of *Serine/threonine kinase 35* (*STK35*) and increase the levels of this transcript without requirement for importin β1. The nuclear accumulation of importin α occurred following exposure to stresses which decreased intracellular ATP levels and was followed by non-apoptotic cell death. Hence the gene regulatory function of nuclear importin α can direct cell fate. There are now several reports of nuclear-localized importin α proteins in diverse cellular states, including cancer. Here we discuss the physiological significance of this novel functional capacity of nuclear importin α relationship to a variety of cellular states and fates.

Keywords: importin α, KPNA2, nuclear transport, stress, STK35, ATP, non-apoptotic cell death

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A Novel Role for Importin α Proteins in Gene Regulation

We found that HeLa cells overexpressing nuclear importin α2 exhibited downregulation of transcripts encoded by 62 genes, including 22 encoding replication-dependent histones, as well as selective upregulation of only two transcripts, including *Serine/threonine kinase 35* (*STK35*).[1] The contrast between the large numbers of downregulated mRNAs with the small number identified as upregulated suggested to us that importin α2 can effectively suppress gene expression through chromatin binding. We hypothesize that this occurs through importin α interaction with the cNLSs in karyophilic proteins such as transcription factors, since the cNLS has been shown to overlap with DNA binding regions in some cargo proteins.[2-4] Thus we predict that, in circumstances when importin α accumulates in the nucleus, certain transcription factors interact with importin α via their cNLS and this binding compromises or changes their transcriptional activities. Because the apparent numerical difference between the number of up- and downregulated genes suggests that nuclear importin α generally acts as a suppressor for transcription factors, and in case of *STK35*, it operates by suppressing the activity of some protein that inhibits transcription. In support of this, we found that the region ≥ 1 kbp upstream of the first exon of *Stk35* served a repressor function for the core promoter.[1] These data suggest that importin α inhibits the suppressor for the *STK35* core promoter, resulting in its enhanced activity.

A New Perspective on Non-Apoptotic Cell Death Associated with Nuclear Importin α

The physiological significance of changes of nucleocytoplasmic transport under stress conditions has been linked to perturbed protein shuttling within signaling cascades, structural modifications of transport machinery, including the nuclear pore

complex (NPC), and evocation of cell death by apoptosis.[5-7] However, there is relatively little discussion about the mechanisms by which changes in cellular metabolism arising from stress, its associated alterations in nucleocytoplasmic transport, and the subsequent impact on cell fate.[8,9] We previously reported that intracellular ATP levels decreased following exposure to all tested stresses: UV-irradiation, heat shock and hydrogen peroxide, and this caused the Ran gradient to collapse.[10] This finding is in agreement with a previous report that cellular ATP depletion following exposure to 2-deoxyglucose and sodium azide leads to a decrease in free RanGTP which is followed by nuclear accumulation of importin α.[11] Depletion of cellular ATPs itself has been known to induce necrosis or caspase-independent cell death, but not apoptosis, because of the high levels of ATP required for caspase activation.[12,13] In addition, apoptosis requires active nuclear transport mediated by importin α and is dependent upon a Ran gradient and intact NPCs.[14] Thus several observations support our hypothesis that blocking the classical nuclear transport pathway, including by induced nuclear accumulation of importin α under conditions of ATP depletion, results in the inhibition of apoptosis and promotion of non-apoptotic cell death. Taken together with the ability of STK35 to enhance caspase-independent cell death under oxidative stress,[1] it becomes evident that the combined outcomes of both deficient classical nuclear transport and transcriptional modulation by nuclear-localized importin α direct cell fate toward a cell death pathway that bypasses apoptosis, such as necrosis, upon stress exposure. These findings reveal a new mechanistic approach to understanding how non-apoptotic cell death is elicited by a decrease in intracellular ATP in cells under stress, through the re-distribution of importin α into the nucleus (**Fig. 1**).

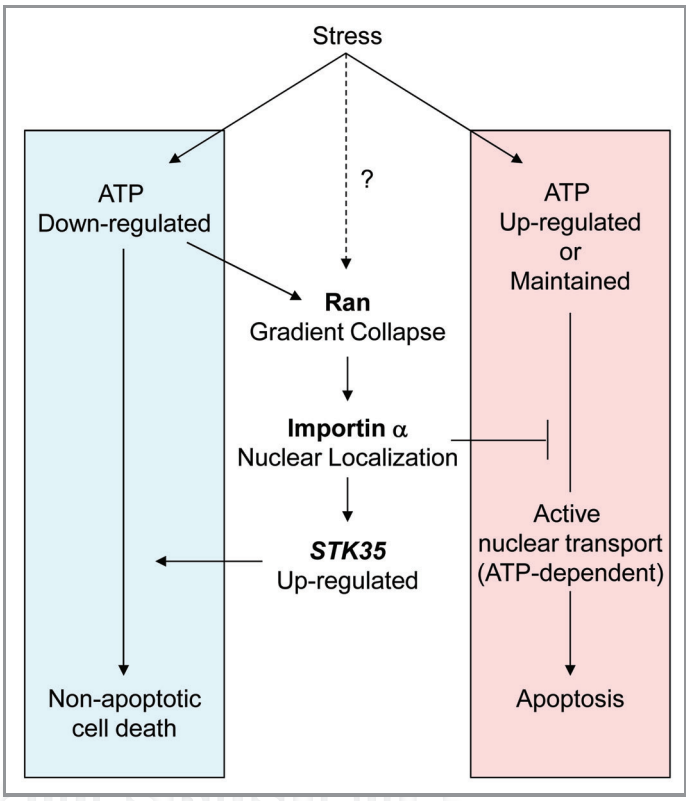

Figure 1. Schematic model for mode of cell death induced by stress in response to depleted intracellular ATP. Cellular stresses which deplete intracellular ATP induce a Ran gradient collapse and importin α accumulates in the nucleus. This leads to a block in classical nucleocytoplasmic protein transport via the importin α/β1 pathway. Nuclear importin α functions to elevate *STK35* transcription and promotes non-apoptotic cell death in oxidative stress. The Ran gradient collapse may be induced by both ATP depletion and through modulated activity of Ran-related proteins, such as RCC1, in some stress conditions.[9]

Additional Physiological Importance of Importin α Nuclear Localization

Is the nuclear accumulation of importin α restricted to stress conditions? C. elegans importin α proteins, particularly IMA-1 and -2, were detected in the nucleoplasm of germ cells.[15] In Drosophila, all three importin αs exhibit nuclear accumulation in a stage-specific manner during spermatogenesis.[16] In mammals, importin α4, but not its close subfamily member importin α3, is predominantly nuclear in the adult testis, with a striking nuclear signal evident in pachytene spermatocytes and round spermatids.[17,18] In addition, the importin α4 protein exhibits nuclear localization in the murine embryonic stem (mES) cells in undifferentiated, but not differentiated, stages.[19] These observations suggest that nuclear-localized importin α proteins serve key roles in cell fate choice between maintenance of pluripotency and differentiation.

Recently, a novel importin α family member was identified, referred to as karyopherin α7 (KPNA7) in human, mouse and cattle.[20-22] KPNA7 is closely related to importin α2 and localized in the nucleus in mouse oocytes and zygotes as well as in HeLa cells.[20,21] Interestingly, a mutant *Kpna7* gene caused abnormal expression of chromatin modification-associated genes and also induced epigenetic modification of histone H3K27me3.[21] These observations bear a striking correlation to our finding that nuclear importin α2 causes downregulation of mRNAs encoding replication-dependent histones[1] and highlight the need to gain a precise understanding of the genomic and chromatin-associated modifications effected by nuclear importin α in the nucleus.

Of direct relevance to human disease, breast cancer cells exhibit the remarkable expression and nuclear localization of human karyopherin α2 (KPNA2, ortholog of mouse importin α2), and this may

be significantly associated with patient survival rates.[23-26] High expression and nuclear localization of KPNA2 was also observed in lung tumor tissues,[27] esophageal squamous cell carcinoma,[28] bladder cancer[29] and prostate cancer.[30] Moreover, increased expression and elevated nuclear accumulation of importin α5 and importin α7 have been reported in tubular and glomerular cells of diabetic rats.[31]

Collectively these reports highlight the potential contribution of nuclear importin α to various cellular events, each of which might involve a different substrate specificity, reflect cell-specific expression patterns and effect distinct transcriptional outcomes. Our findings should encourage investigations of additional functions for importin α in a variety of cellular states and fates.

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