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*Published in:*  
Biochimica et Biophysica Acta - Reviews on Cancer

*DOI:*  
[10.1016/j.bbcan.2015.09.001](https://doi.org/10.1016/j.bbcan.2015.09.001)

2015

[Link to publication](#)

*Citation for published version (APA):*  
Kazi, J. U., Kabir, N. N., & Rönnstrand, L. (2015). Brain-Expressed X-linked (BEX) proteins in human cancers. *Biochimica et Biophysica Acta - Reviews on Cancer*, 1856(2), 226-233.  
<https://doi.org/10.1016/j.bbcan.2015.09.001>

*Total number of authors:*  
3

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## **Brain-Expressed X-linked (BEX) proteins in human cancers**

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### **Abstract**

The Brain-Expressed X-linked (BEX) family proteins are comprised of five human proteins including BEX1, BEX2, BEX3, BEX4 and BEX5. BEX family proteins are expressed in a wide range of tissues and are known to play a role in neuronal development. Recent studies suggest a role of BEX family proteins in cancers. BEX1 expression is lost in a subgroup of patients with acute myeloid leukemia (AML) and chronic myeloid leukemia (CML). Expression of BEX1 controls cell surface receptor signaling and restores imatinib response in resistant cells. BEX2 is overexpressed in a group of breast cancer patients and also in gliomas. Increased BEX2 expression led to enhanced NF-κB signaling as well as cell proliferation. Although BEX2 acts as tumor promoter in a subset of breast cancer, BEX3 expression displayed an opposite role. Overexpression of BEX3 resulted in inhibition of tumor formation in breast cancer mouse xenograft models. The role of BEX4 and BEX5 in cancer has not yet been defined. Collectively this suggests that BEX family members have distinct roles in cancers. While BEX1 and BEX3 act as tumor suppressors, BEX2 seems to act as an oncogene.

### **Introduction**

Signals from extracellular stimuli, such as growth factors, evoke diverse cellular responses including cell survival, proliferation and apoptosis. Cell surface receptors transduce signals from growth factors,

and these signals are tightly controlled by intracellular proteins. These proteins propagate or inhibit receptor downstream signaling by different mechanisms. For example, ubiquitin ligases or phosphatases mainly turn off receptor signaling by destabilizing the receptor or removing phosphorylation which is the hallmark of the activation of many receptors. Kinases such as SRC family kinases (SFKs) are involved in propagating receptor signals to other signaling proteins. Adaptor proteins also play important roles in receptor signaling. The Brain-Expressed X-linked (BEX) gene family appears to be a new class of proteins that regulate signals from different cell surface receptors. In the rat brain, these genes account for more than 12% of the expressed sequence tags [1]. BEX family proteins are expressed in a wide range of tissues and play diverse roles. In this review we discuss our current understanding of BEX family proteins.

### **BEX genes and proteins:**

The human BEX family includes five proteins, BEX1, BEX2, BEX3 or p75NTR-associated cell death executor (NADE), BEX4 and BEX5. Although, BEX family genes are well conserved in mammals [2], the human BEX5 homolog is absent in mice, while BEX6 was identified as a mouse specific gene [3]. BEX proteins display considerable sequence similarity between different species (Fig. S1) and all BEX proteins contain a characteristic BEX domain (Fig. 1). Beside BEX family proteins, BEX domains are also found in another class of proteins which are known as transcription elongation factor A (SII)-like (TCEAL) [4, 5]. The function of this domain is still largely unknown. However, recent studies suggest that BEX domain-containing proteins are involved in the control of cellular growth [6-8]. All BEX genes cluster close to each other and human BEX genes were mapped to the Xq22 chromosome. BEX1 and BEX2 contain a conserved motif, Ser-Leu-Arg, near the C-terminus [9], which is known to be phosphorylated by protein kinase C (PKC) [10]. PKC is a family of 10 serine/threonine protein kinases that has been implicated in many cancers [11-15]. Other serine/threonine protein kinases can also phosphorylate BEX proteins. A more recent study identified a serine phosphorylation site in mouse BEX1 which is phosphorylated by the serine/threonine protein kinase AKT [16].

**BEX1:** Initially BEX1 was described as a gene downregulated following retinoic acid treatment of F9 murine teratocarcinoma cells [17]. This gene was also highly expressed in parthenogenetic blastocysts [18, 19]. BEX1 is transiently expressed during the initial stage of embryonic development and are also expressed during the preimplantation stage in blastocysts of mouse embryos [18, 20, 21]. Later studies identified high BEX1 expression in human brain, pancreas, testis, and ovaries [9], while human heart, placenta, liver, kidney, spleen, thymus, prostate, small intestine, colon, thyroid, spinal cord, and adrenal gland express comparatively low levels of BEX1 mRNA [9]. Thus, BEX1 is expressed in a wide range of tissues. Another study demonstrated BEX1 expression in the central nervous system with high levels in the pituitary, cerebellum, and temporal lobe [1].

**BEX2:** BEX2 was initially described as BEX1 but was later changed to BEX2 since there was already another BEX1 described in another paper [22, 23]. Exclusive expression of BEX2 was reported in the pancreas, kidney, liver, adrenal gland and testis but not in normal cells of hematopoietic tissues [22]. Human BEX2 was identified in fetal brain and a role in embryonic development has been suggested [24]. Expression was upregulated in MLL mutant AML cell lines compared to MLL wild-type AML cell lines suggesting a role in this particular type of hematological cancers. In addition to hematological cancers BEX2 expression was detected in breast cancer cell lines such as MCF-7, MDA-MB-231, T-47D, BT-474 and SK-BR-3 but not in MDA-MB-453 cell lines [25]. BEX2 expression was also found to be upregulated in glioma tissue [26]. Similar to BEX1 expression, BEX2 displayed expression in the central nervous system with high levels in the pituitary, cerebellum, and temporal lobe and also in the liver [1].

**BEX3:** The first identified BEX family gene was human BEX3, originally known as HGR74. Expression of HGR74 was described in human testis, prostate, seminal vesicles, and ovarian granulosa cells [27]. Mouse BEX3 was isolated from an embryo cDNA library by a yeast two-hybrid screening and named as NADE as it was identified as a p75NTR-associating protein [28]. Differential expression of mouse BEX3 has been described. Mouse brain, heart, and lung express the highest level of BEX3 mRNA while stomach, small intestine, and muscle tissues express comparatively low levels of BEX3 mRNA [28]. BEX3 protein was detected in PC12 and PCNA cells only after treatment with proteasomal inhibitors suggesting that BEX3 expression is regulated by proteasomal degradation [29]. Expression of BEX3 was described in pillar cells [30] and considerable higher expression was detected during mouse embryonic development [31].

**BEX4 and BEX5:** Unlike other BEX family genes, BEX4 and BEX5 have not been studied well. While human BEX5 is widely expressed in many tissues, high BEX4 expression has been reported in heart, skeletal muscle, and liver [1].

#### **Stability and subcellular localization:**

BEX proteins display differential subcellular localizations. While human BEX1 was found to be localized to the cytosolic compartment [8], rat BEX1 mainly localizes to the nucleus [1]. Human and rat BEX1 proteins display considerable sequence difference showing only 67% sequence similarity, thus explaining the difference in localization patterns. This is probably due to differential affinity to associating proteins that determine localization. Rat BEX3 and human BEX5 localizes to the cytoplasm and rat BEX2 and rat BEX4 localizes both to nucleus and cytoplasm [1]. A short sequence of the nuclear export signal (NES) is present in mouse BEX3 suggesting the presence of BEX3 protein in both nucleus and cytosol [28]. The stability of BEX proteins has been studied. Mouse and

rat BEX3 are rapidly degraded in the proteasomes [1, 28]. Two boxes that are sequences targeted for ubiquitination have been described [28]. It is also evident from experiments that BEX3 protein could only be detected in PCNA and PC12 after 3 hours inhibition of proteasome activity by proteasome inhibitors (ALLN, PSI, and MG132) [28]. Rat BEX4 and human BEX5 are also degraded in the proteasome, but BEX1 and BEX2 were resistant to proteasomal degradation [1].

### **The role of BEX proteins in normal cells**

BEX1 has been identified as one of an important gene required for muscle differentiation [32]. BEX1 associates with calmodulin (CaM) in a  $\text{Ca}^{2+}$ -dependent manner [32]. Since  $\text{Ca}^{2+}$ -dependent CaM signaling is important for skeletal muscle generation [33-35] and BEX1 expression was upregulated after cardiotoxin (CTX) treatment [32], it has further been suggested that BEX1 plays a role in skeletal muscle generation. Mice lacking BEX1 expression appear to develop normally and are fertile, except for altered muscle regeneration [36]. During liver development higher BEX2 levels were identified in stem or progenitor cell populations suggesting that BEX2 is a novel marker for stem or progenitor cells during liver development [37]. Although BEX2 served as a novel marker of hepatoblasts in the mid-fetal liver, loss of BEX2 expression did not influence cell proliferation or differentiation. Furthermore, mice deficient of BEX2 were viable and fertile under normal growth conditions and did not show any obvious phenotypic abnormalities [37]. Although BEX2 is apparently not involved in liver development, another study suggested that human BEX2, but not murine BEX1 or BEX2, associates with LIM-domain containing transcriptional factor (LMO2) [24], leading to enhancement of NSCL2-dependent transcriptional activity which is required for embryonal development.

BEX proteins have been suggested to play a role in neuronal development. The BEX1, BEX2 and BEX3 proteins interact with the olfactory marker protein (OMP) [38-40]. Expression of OMP is the hallmark of mature vertebrate olfactory receptor neurons (ORNs) [41, 42]. Elevated BEX1 expression was detected in spinal motor neurons (MNs) of peripheral myelin protein 22 (Pmp22) mutants and in mutant mice that develop MN degeneration [43, 44]. Upregulation of BEX1 expression was observed in spinal cord MNs after axonal injury [45]. Furthermore, BEX1 knockout mice displayed a defect in recovery from sciatic nerve injury [46] suggesting that in addition to skeletal muscle regeneration, BEX1 plays a role in neuronal regeneration.

### **Epigenetic suppression of BEX expression**

Epigenetic suppression of tumor suppressor genes is a common phenomenon in human cancers [47]. Alterations of chromatin structure through promoter hypermethylation is one of the common mechanism of epigenetic suppression [48]. Histone deacetylation, histone methylation, and other histone modifications also play important roles in this process [49]. DNA methyltransferase and histone deacetylase (HDAC) inhibitors such as Trichostatin A (TSA) and 5-aza-2'-deoxycytidine (5-

AzaC) are widely used to define the role of epigenetic modification in cancers [50, 51]. Treatment of glioma cells with TSA or 5-AzaC resulted in strong induction of BEX1 and BEX2 expression suggesting an epigenetic suppression of its expression in glioma which was further shown to be mediated through promoter methylation and histone modification [52]. Methylation of the BEX1 promoter was associated significantly ( $p < 0.0001$ ) with oral squamous cell carcinoma (OSCC) and were detected in 75% (42/56) of the samples [53]. TSA and 5-AzaC treatment substantially elevated BEX2 expression in MLL wild-type AML cells suggesting that BEX2 is epigenetically suppressed in AML as well [23]. Hypermethylation of BEX2 promotor region has been reported in MLLwt AML cell lines which could be restored by demethylating agents and inhibitors of histone deacetylases [54]. Therefore, it is likely that expression of BEX1 and BEX2 was epigenetically suppressed due to hypermethylation of the promoter region.

### **BEX proteins in neurotrophin receptor signaling**

The neurotrophins are neuronal growth factors involved in the development, maintenance, survival, differentiation and apoptosis of the nervous system [55]. Nerve growth factor (NGF) is the most studied neurotrophin, while others include brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3, and NT-4/5 [56]. The p75 neurotrophin receptor (p75NTR) and the tropomyosin-related kinase (TRK) family of receptors are known receptors of neurotrophins. The transmembrane receptor p75NTR is a member of the tumor necrosis factor receptor family which is involved in cell survival and apoptosis [57]. Rat primary oligodendrocytes expressing higher levels of p75NTR were apoptotic in response to NGF-induction [58]. Mukai et al. showed that NGF treatment elevated BEX3 mRNA and protein levels in oligodendrocytes [28] suggesting a possible role of BEX3 in NGF-induced apoptosis. Zinc exposure also induced p75NTR and BEX3 in cortical culture resulting in cell death [59]. Furthermore dopamine responsive gene-1 (DRG-1) binds with BEX3 *in vivo* and *in vitro* in the cytoplasmic compartment and BEX3 expression blocked DRG-1 induced cell proliferation of PC12 cells also suggesting a tumor suppressive role [60]. In response to NGF, BEX3 and p75NTR complex was immunoprecipitated from proteasome inhibitor treated PC12 cell lysates [28]. BEX3 associated with the death domain of p75NTR through 81-106 residues [28, 61]. Similar to NGF treatment, BEX3 and p75NTR complex was co-immunoprecipitated from cortical neurons [59]. This association was abrogated in the presence of an antibody that blocked p75NTR function suggesting that zinc-induced BEX3-p75NTR association is regulated by NGF and that NGF is involved zinc-induced neuronal cell death. In addition p75NTR BEX3 was found to be associated with the adaptor protein 14-3-3 $\epsilon$  which was essential for NGF-induced p75NTR/BEX3 mediated apoptosis in PC12nnr5 cells and oligodendrocytes [62]. Apoptosis is mediated through activation of several caspases. At least three caspases including caspase-1, caspase-2 and caspase-3 were reported to be activated during NGF-induced p75NTR-mediated oligodendrocyte cell death [58]. Activation of caspase-2 and caspase-3 was observed in BEX3 expressing cells in response to NGF suggesting that BEX3 mediated apoptosis

is mediated through activation of caspases [28]. In addition, BEX3 binds to hamartin, a protein expressed by the tuberous sclerosis complex 1 (TSC1) gene [63]. Sporadic mutations in TSC1 is associated with a rare multi-system genetic disease tuberous sclerosis causing benign tumors in the brain or other organs [64]. Association of hamartin, and BEX3 were required for NGF-induced apoptosis in PC12h cells, as siRNA-mediated knockdown of TSC1 gene in BEX3 overexpressing cells were protected from NGF-induced apoptosis [63]. Another class of NGF receptor TrkA was associated with BEX3 through the juxtamembrane domain of the receptor. Association was independent of NGF-induction, while NGF increased association in PC12 cells [65]. TrkA and BEX3 co-expressed in embryonic rat dorsal root ganglia (DRG) neurons and also form complex further suggesting a possible role BEX3 in neuron development. Like BEX3, BEX1 was also found to be associated with p75NTR in PC12 cells and the interaction was independent of NGF stimulation [16]. BEX1 expression overlaps with that of p75NTR in developing nervous system and in vascular and mesenchymal structures and overexpression of BEX1 in PC12 cells resulted in cell cycle arrest and neuronal differentiation without affecting cell proliferation suggesting a role of BEX1 in development of nervous system. Furthermore, overexpression of BEX1 in PC12 cells was associated with reduced NF $\kappa$ B activity in response to NGF as well as elevated differentiation capacity, while knockdown of BEX1 potentiated NF $\kappa$ B activity in response to NGF [16]. Therefore, it has been suggested that both BEX1 and BEX3 interact with p75NTR and regulate NGF-induced apoptosis and differentiation in neural tissues through NF-  $\kappa$ B (Fig. 2).

#### **The role of BEX proteins in breast cancer:**

The role of NGF has been studied in the context of breast cancer. NGF-stimulation induces proliferation of MCF-7 and MDA-MB-231 breast cancer cell lines [66] and protects MCF-7 cells from ceramide analog-induced apoptosis [67, 68]. However, NGF did not display an effect on normal breast epithelial cells. Breast cancer cell lines MCF-7, T47-D, BT-20, and MDA-MB-231 cells express NGF receptor p75NTR and TrkA suggesting that NGF-induced biological effects in breast cancer are mediated through these two receptors [67]. Neutralizing antibodies and pharmacological inhibitors against p75NTR effectively rescued ceramide analog-induced apoptosis but did not affect cell proliferation in response to NGF. While TrkA inhibitors were effective against NGF-induced cell proliferation, they did not modulate apoptosis [67]. Therefore, NGF-induced biological effects in breast cancer are highly dependent on receptor expression profile and activity. Activation of the NF-  $\kappa$ B pathway by NGF through p75NTR is involved in protection against ceramide analog-induced apoptosis [67, 68]. A subset of estrogen receptor positive breast cancer samples displayed elevated expression of BEX1 and BEX2 mRNA [69]. Furthermore, BEX2 expression was elevated in MCF7 cells upon estrogen treatment and over-expression of BEX2 protected MCF7 cells from ceramide analog-induced apoptosis suggesting that BEX2 mimics the effects of NGF treatment. In addition, knockdown of BEX2 impaired the anti-apoptotic response to NGF-treatment [25, 69] indicating that



BEX2 is required in order for p75NTR to transduce the signal from NGF to NF- $\kappa$ B. BEX2 expression also protected cells from tamoxifen-induced apoptosis. Although BEX2 expression protected cells from apoptotic responses, it did not contribute to NGF-induced cell proliferation further suggesting that BEX2 acts a component of the p75NTR signaling pathway [69]. In addition to regulating the NF- $\kappa$ B activity, BEX2 protected the breast cancer cells against mitochondrial apoptosis by inducing BCL2 and BAX phosphorylation [25]. BEX2 depletion potentiated Protein Phosphatase 2A (PP2A) activity in breast cancer cells, explaining how BEX2 protects cells from mitochondrial apoptosis. Ceramide treatment induced BEX2 expression in MCF7 and MDA-MB-231 cell lines [69, 70]. The BEX2 promotor has binding sites for c-Jun and p65 which are also known to be a regulator of ceramide signaling [71]. BEX2 expression is required for the activation of p65 and c-Jun as well as JNK kinase activity [70]. Furthermore c-Jun mediated induction of cyclin D1 and cell proliferation in breast cancer cell lines was partially dependent on BEX2 expression [70]. Therefore, it is likely that BEX2 acts in a feedback loop where expression of BEX2 is regulated by p65 and c-Jun through ceramide treatment and then BEX2 expression results in impaired PP2A activity increasing p65 and c-Jun activity. Furthermore BEX2 expression is correlated with ErbB2 expression and like ceramide ErbB2 induces transcriptional activation of BEX2 expression through c-Jun activation [72]. Upregulation of BEX2 expression in turn increased c-Jun-mediated induction of ErbB2 in MCF-7 cells [72] suggesting that another feedback loop between BEX2 and ErbB2 is involved in breast cancer.

A gene expression profiling study with HER2-negative breast tumors identified five genes, including SERPINA6, BEX1, AGTR1, SLC26A3, and LAPTM4B, as a markers of chemotherapy resistance [73]. However, the function of BEX1 in breast cancer still remains unknown. BEX3, another BEX protein, was reported to be highly expressed in human endocrine-related organs and embryonic murine tissues demonstrating a role in breast cancer. Overexpression of BEX3 in human breast cancer cells MDA-MB-231 led to dramatic suppression of *in vivo* tumor formation [74]. BEX3 associates with SMAC and promotes TNF-induced apoptosis in MCF-7 breast cancer cells [75]. Thus, BEX2 and BEX3 play opposite roles in breast cancer, where BEX3 acts as a tumor suppressor and BEX2 acts a tumor promotor.

### **BEX proteins in gliomas:**

Malignant gliomas are the most common and aggressive brain tumors [76]. Our current understanding of gliomas pathogenesis suggests that loss of function of tumor suppressor genes and gain of function or activation of oncogenes are involved in activation of oncogenic signaling pathways [77-79]. A recent study demonstrated that expression of both BEX1 and BEX2 was lost in human glioma cell lines and primary patient samples [52]. However, another report examining 32 gliomas vs 15 non-tumor tissues showed that BEX2 expression was 2.73 fold upregulated in glioma [26]. Selective depletion of BEX2 expression in glioma cells led to reduction of U251 cell proliferation, while

overexpression resulted in elevated cell proliferation [26, 80]. Similar to the breast cancer cells, BEX2 expression enhanced p65 expression as well as activation of NF- $\kappa$ B pathway in the U251 cells. Furthermore, BEX2 expression promoted U251 and U87 glioma cells migration and invasion by regulating N-cadherin and  $\beta$ -catenin expression [81, 82]. Therefore, it is likely that BEX2 acts as tumor promotor in gliomas.

### **BEX proteins in acute myeloid leukemia (AML)**

Acute myeloid leukemia (AML) is a heterogeneous disease of blood that originates in bone marrow. The receptor tyrosine kinase FLT3 is expressed in almost all AML patient and is mutated in as high as 35% of AML patients. FLT3 is a member of type III receptor tyrosine kinase family (also called the platelet derived growth factor receptor (PDGFR) family) [83]. A small portion of acute lymphoblastic leukemia (ALL) patients also carry mutations of FLT3 [84]. Signaling downstream of FLT3 is tightly controlled by various signaling proteins which associate with FLT3 and regulate downstream signaling by propagating the signals from extracellular stimuli or diminish the signal by destabilizing the receptor. For example, association of GRB10, SLAP, SYK and CSK resulted in enhanced FLT3 signaling, while the association of SOCS6, SOCS2 and LNK suppressed FLT3 signaling by destabilizing the receptor [85-95]. BEX1 expression was found to be down-regulated in a group of FLT3-ITD positive AML patients [8]. Overexpression of BEX1 in mouse pro-B cells or myeloid cells expression FLT3-ITD resulted in selective inhibition AKT phosphorylation as well as cell proliferation, colony and tumor formation. In addition, loss of BEX1 expression was correlated with poor overall survival in FLT3-ITD positive AML patients [8]. Based on these data, it is suggested that BEX1 acts as a tumor suppressor in FLT3-ITD positive AML.

### **BEX proteins in chronic myeloid leukemia (CML)**

Chronic myeloid leukemia (CML) is a hematological cancer that causes marked increases in white blood cells and platelets [96]. CML is caused by the fusion of parts of the BCR gene with parts of the ABL gene due to chromosomal translocation. BCR/ABL fusion protein has stronger kinase activity than the wild-type ABL kinase and is constitutively active. Therefore, imatinib, a selective BCR/ABL kinase inhibitor, displayed promising results in CML treatment. However, long-term use of imatinib results in some cases to resistance to the drug due to mainly acquired mutations in the inhibitor binding site [97]. Other mechanisms have also been suggested including amplification of BCR/ABL gene, expression of other oncogenes or multidrug resistance genes, and loss of drug transporter proteins [96-100]. A study with K562 CML cell line suggested that deregulation of BEX1 could be a new mechanism of resistance in CML. The long-term treatment with imatinib resulted in loss of BEX1 expression in the BCR/ABL positive K562 leukemia cell line rendering it resistant to imatinib [101]. The inhibitor 5'-Aza-2'-deoxycytosine did not restore BEX1 expression in resistant cell lines, suggesting that downregulation of BEX1 is not related to the hypermethylation of BEX1 promotor

region. Overexpression of BEX1 could effectively restore imatinib sensitivity in the resistant cells [101]. The BEX1 expression did not block BCR/ABL-induced AKT or NF $\kappa$ B activation, but it activated the JNK pathway. BEX1 was demonstrated to activate caspase3/7 through the non-classical pathway in the presence of imatinib. PCDH10 could be co-immunoprecipitated with BEX1 and reported to be downregulated in cells that had lost BEX1 expression. Moreover, depletion of PCDH10 resulted in imatinib-resistance in K562 cells [101] suggesting that PCDH10 is involved in BEX1-induced apoptosis in response to imatinib. BEX1 association with BCL2 and localization to the mitochondria has been shown to induce apoptosis. Through these mechanisms, CML cells that has lost BEX1 expression are resistant to imatinib-induced apoptosis [102].

### **BEX proteins in other cancers:**

BEX proteins are also implicated in many other cancers. For example, BEX1 was found to be upregulated in neuroendocrine tumors [103]. BEX1 was identified as the most frequently methylated genes (27/40 cases) in pediatric intracranial ependymoma [104]. Ectopic expression of BEX1 significantly suppressed cell proliferation and colony formation in pediatric ependymoma during short-term cell culture [104]. The mouse teratocarcinoma cell line F9 and the human ovarian carcinoma cell line PA-1 express BEX3 where BEX3 was found to be associated with mitochondria [105] and probably regulates mitochondrial function.

### **Conclusions**

BEX family proteins display diverse functions in normal cells as well as in human cancers. Several BEX family proteins are involved in different signaling pathways and play important roles by interacting with specific signaling proteins [106]. BEX1 and BEX3 are involved in the control of mitogenic signaling from p75NTR and induce apoptosis in response to NGF, suggesting a tumor suppressive role of BEX proteins. Expression of BEX family proteins is regulated through epigenetic modification where hypermethylation of the promotor region resulted in suppression of BEX expression. Since BEX1 and BEX3 act as tumor suppressors in several cancers including AML, CML, breast cancer and ependymoma (Table 1), patients with lower BEX1 and BEX3 expression will most likely benefit from treatment with epigenetic modifier drugs that enhance BEX expression. Although BEX1 and BEX3 play similar roles in cancer, BEX2 expression is upregulated in a subset of breast cancer as well as in glioma patients and expression of BEX2 resulted in enhanced cell proliferation suggesting a possibility of using BEX2 as a target for therapy in these cancers. While BEX1 and BEX2 display considerable homology in their protein sequence, current studies suggest opposite roles of these proteins. A possible explanation to this discrepancy could be that the affinity for interacting proteins might be influenced by differences in single amino acids in the sequence and therefore different despite a high degree of similarity. Collectively, our current understanding suggests that BEX family members play distinct roles in cancer. Although current studies suggest a tumor

suppressive role of BEX1 and BEX3, and tumor-promoting role BEX2 in few cancers, more studies are needed before targeting this family protein in cancer. Furthermore, the role of other BEX family members such as BEX4 and BEX5 remains by and large unknown.

**Acknowledgements:** This research was funded by Kungliga Fysiografiska Sällskapet i Lund (JUK), Ollie and Elof Ericssons Stiftelse (JUK), Åke-Wiberg Stiftelse (JUK), Lars Hiertas Minne Stiftelse (JUK), Stiftelsen Olle Engkvist Byggmästare (JUK), Harald Jeanssons Stiftelse and Harald och Greta Jeanssons Stiftelse (JUK), Swedish Childhood Cancer Foundation (JUK), The Crafoord foundation (JUK), Stiftelsen Clas Groschinskys Minnesfond (JUK), Swedish Cancer Society (LR), Swedish Research Council (LR) and Gunnar Nilssons Cancer Foundation (LR). JUK is a recipient of an Assistant Professorship (forskarassistenttjänst) grant from the Swedish Childhood Cancer Foundation.

**Conflicts of interest:** The authors declare no conflict of interests.

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### Figure legends:

Fig. 1: BEX family proteins: All human BEX family proteins contain a characteristic BEX-domain. BEX2 was initially named as BEX1 and similarly BEX1 was also named as BEX2. BEX3 is well known as NADE.

Fig. 2: BEX family proteins in NGF signaling: NGF activates p75NTR and TrkA resulting in activation of cell proliferation and survival through activation of NF- $\kappa$ B, AKT and MAPK signaling pathways. Additionally NGF induces apoptosis pathway through JNK pathway. Association of BEX1 with p75NTR resulted in inhibition of NGF-induced NF- $\kappa$ B activation. BEX3 associates with p75NTR and recruits 14-3-3 $\epsilon$  and Hamartin and also activates apoptosis pathway. Association of BEX3 to the receptor accelerates degradation of BEX3 in proteasomes.

Table 1: BEX family members in human cancer

BEX member	Cancer type	Role in cancer	Reference
BEX1	Oral squamous cell carcinoma	Methylation of the promoter	[53]
	Breast cancer	Elevated expression of mRNA	[69]
	AML	Deregulated and loss of expression resulted in poor overall survival	[8]
	CML	Loss of expression resulted in imatinib-resistant	[101]
	Neuroendocrine tumors	Upregulated	[103]
	Pediatric intracranial ependymoma	Overexpression suppressed cell proliferation and colony formation	[104]
BEX2	Breast cancer	Elevated expression of mRNA, anti-apoptotic.	[25, 69]
	Glioma	Upregulated, tumor promotor	[26, 80]
		promoted U251 and U87 glioma cells migration and invasion	[81, 82]
BEX3	Breast cancer	Suppression of in vivo tumor formation, Pro-apoptotic	[74, 75]



FIGURE 1

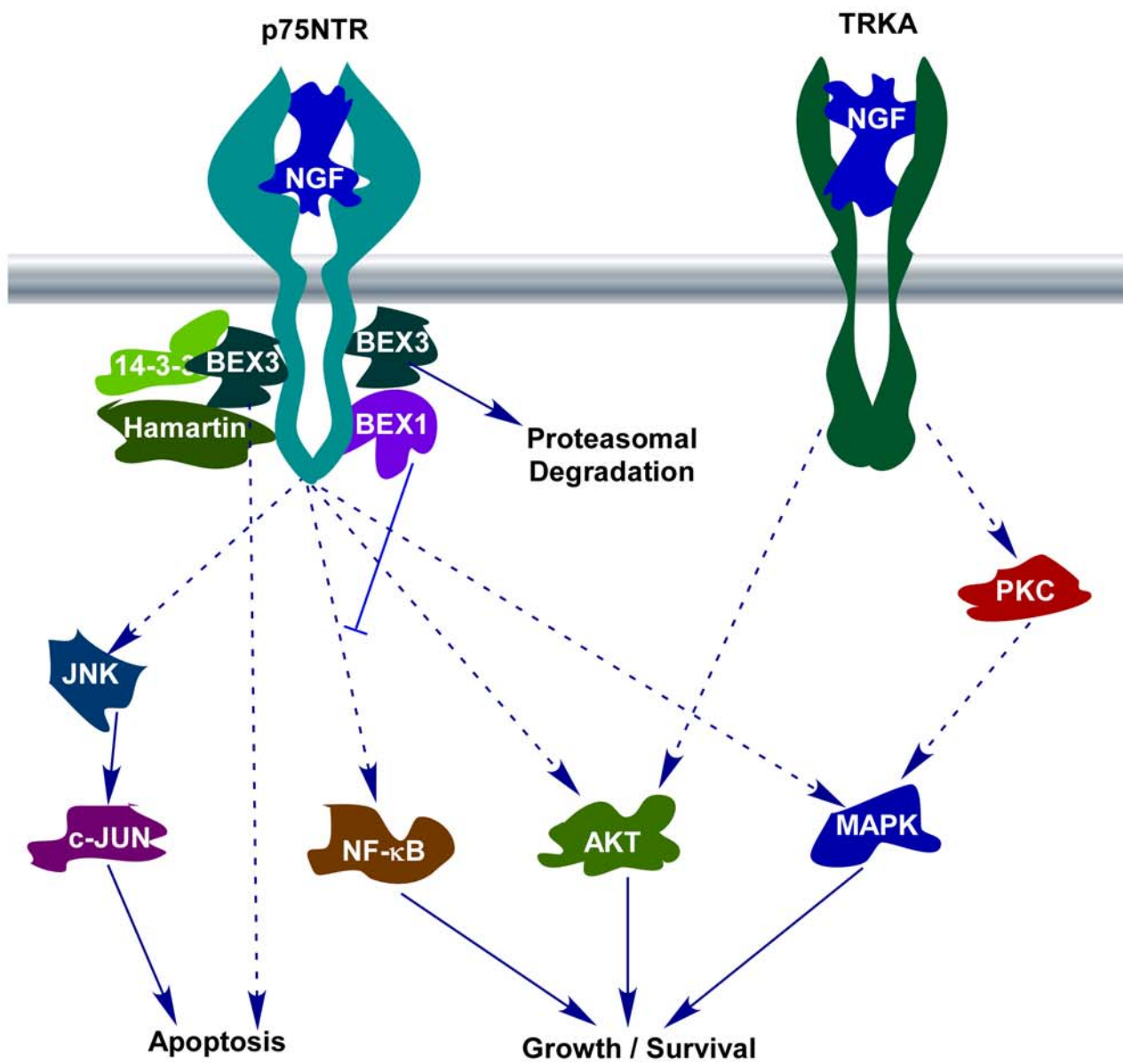


FIGURE 2

**Figure S1:** Sequence alignment of BEX proteins.

