Differential mRNA Expression of AURKA, AURKB and AURKC in Neuroblastoma Cell Lines

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Abstract

Neuroblastoma is the most common extracranial solid cancer in childhood and the most common cancer in infancy.There are three cell types:the neuroblastic (N) phenotype, the highly substrate-adherent (S) phenotype and the intermediate(I) phenotype. Aurora kinase family plays an important role in mitosis. Recent study showed that Aurora kinase family has close relationship with neuroblastoma RT-PCR method was used to measure the mRNA expression of Aurora kinase A,B,and C in neuroblastoma cells exhibiting either N ,I or S phenotypes. Aurora A is expressed at higher level in I type cells. Aurora C is expressed the lowest in the I cell type. Due to the increasing relevance of abberations in the aurora kinase dependent pathway in human tumorigenesis, further studies are warranted to study the level and activity of other components of this pathway .

Introduction

Neuroblastoma is the most common extracranial solid cancer in childhood and the most common cancer in infancy, with an annual incidence of about 650 new cases per year in the US. Close to 50 percent of neuroblastoma cases occur in children younger than two years old. (eMedicine - Neuroblastoma : ArticlebyNormanJ Lacayo, MD.）It is a neuroendocrine tumor, arising from any neural crest element of the sympathetic nervous system or SNS. It most frequently originates in one of the adrenal glands, but can also develop in nerve tissues in the neck, chest, abdomen, or pelvis.

Aurora kinases are serine/threonine kinase that are essential for cell proliferation. The enzyme helps the dividing cell share its genetic materials with its daughter cells. More specifically, Aurora kinases play a crucial role in cellular division by controlling chromatid segregation.（Hannak E, Kirkham M, Hyman AA, Oegema K (December 2001).) Defects in this segregation can cause genetic instability, a condition which is highly associated with

tumorigenesis. Three Aurora kinases have been identified in mammalian cells to date. Besides being implicated as mitotic regulators, these three members of the mammalian family have generated significant interest in the cancer research field due to their elevated expression profiles in many human cancers . (Marumoto T, Honda S, Hara T, Nitta M, Hirota T, Kohmura E, Saya H (December 2003).)The human Aurora kinases present a similar domain organization, with a N-terminal domain of 39 to 129 residues in length, a protein kinase domain and a short C-terminal domain containing 15 to 20 residues. The N-terminal domain of three proteins share low sequence conservation, which determines selectivity during protein-protein interactions .

As mentioned above, there are three classes of aurora kinases:Aurora A (aka Aurora 2) functions during prophase of mitosis and is required for correct function of the centrosomes (the microtubule organising centres in eukaryotic cells). Aurora B (aka Aurora 1) functions in the attachment of the mitotic spindle to the centromere. Aurora C (AURKC) works in germ-line cells and little is known about its function.

The purpose of this project was to characterize the expression level differences of the Aurora Kinase genes in N type , I type and S type neuroblastoma cell line. These three types of neuroblastoma cells exhibit difference not only in their phenotypes but also their tumorgenicity and ability to metastasize. KCN-83n is an N– type neuroblastoma, which is a malignant neural crest stem cell, SH-EP1 is S-type, exhibiting substrate adherence and is non-tumorigenic while BE(2)-C is I- type,which is in between. Aurora Kinase is up regulated in several cancers. It is therefore important to see if there is a difference of expression in neuroblastoma cell lines.

Materials and Methods

Cell Lines

KCN-83n , SH-EP1 and BE(2)-C cells were kindly provided by Dr. Robert Ross. The cell lines were cultured in DMEM/F12 medium supplemented with 10% fetal bovine serum at 37°C.

Total RNA Extraction

Total RNA from the three neuroblastoma cell samples was isolated using RNeasy Plus Mini Kit following manufacturer's protocol with minor modifications. The pelleted cells were disrupted by adding 600 µl of Buffer RLT Plus and vortexed to obtain a homogenous lysate. The homogenized lysate was transferred to a gDNA Eliminator spin column placed in a 2 ml collection tube, and was centrifuged for 30s at 13,000 rpm. The column was discarded, and the flow-through was saved. 600ul of 70% ethanol was added to the flow-through and mixed by pipetting. One aliquot of the sample was transferred

to an RNeasy spin column placed in a 2 ml collection tube, and was centrifuged for 30s at 13,000 rpm. The flow-through was discarded. The remained aliquot of smaple was transferred to the same RNeasy column and centrifuged for 30s at 13,000 rpm. The flow-through was discarded. 700µl of Buffer RW1 was added to the RNeasy spin column and centrifuged at 13,000 rpm for 30 s. 500µl of Buffer RPE was then added and centrifuged at 13,000 rpm for 30s, and this step was repeated but centrifuged for 2min. The RNeasy spin column was placed in a new 2ml collection tube and centrifuged at 13,000 rpm for 1min. Then the spin column was placed in a new 1.5ml collection tube, and was eluted with 30µl deionized water. Extracted RNA was stored at -80 .

primer design

Primers were designed for AURKA, AURKB and AURKC genes.The regions of the exons the primer spanning was shown in Figure 1. The sequence of primers are shown in table1.

genes	NCBI Acc #	Exon	Primer sequence 5'- 3'	RT-PCR Prod(bp)	Intron between primers
AURKA	NM_003600.2	Exon 1,2	GGACC TGTTA AGGCT ACAGC	509 bp	2.5kb
		Exon 5	CTTCTGAGCTGATGCTCCAC		
AURKA	NM_003600.2	Exon 6	CATGATGCTACCAGAGTCTACC	336 bp	3.7kb
		Exon 8	GAGATCC ACCTT CTCAT CATGC		
AURKB	NM_004217.2	Exon 2	CCAGAAGGAGAACTCCTACC	649 bp	1.8kb
		Exon 7	GTCAGCAATCTTCAGCTCTC		
AURKB	NM_004217.2	Exon 6	GAAGAGCTGCACATTTGACG	424 bp	2.1kb
		Exon 9	GAGCAGTTTGGAGATGAGG		
AURKC	NM_003160.2	Exon 1	CCTGAAGGTTCTCTTCAAGTCG	401 bp	0.9kb
		Exon 5	CCAGTGTCCCACACATTGTC		
AURKC	NM_003160.2	Exon 5	CTGACCTACTGCCATGACAAG	372 bp	1.3kb
		Exon 7	CTGGTATCTGAGAAGCCTGG		

GAPDH	NM-002046	Exon 2,3	GAAGGTGAAGGTCGGAGT	226bp	1.9kb
		Exon 4,5	GAAGATGGTGATGGGATTTC		

RT-PCR

RT-PCR was performed on the total RNA samples prepared from the three neuroblastoma cell samples using the Qiagen OneStep RT-PCR kit. Twenty nanograms of RNA was amplified in 25μl RT-PCRs (5μl 5×RT buffer, 1μl 10mM dNTPs, 0.5μl 10pmol/μl forward primer, 0.5μl 10pmol/μl reverse primer, 1μl enzyme mix, 4μl 5ng/μl RNA and 13μl ddHRT-PCR was performed according to the following protocol: one cycle of 50°C for 30min and 95 °C for 15min, 94 °C for 30s, 56 °C for 30s, and 72°C for 30s, and a final extension of 72°C for 10min followed by a final hold at 4°C. To control for the amount of RNA present in the samples, RT-PCR amplification of GAPDH was performed on all RNA samples for 28 cycles. 5μl of loading dye was added to each RT-PCR product. 8μl of each product was then added to a 1.2% agarose gel, and electrophoresis was performed at 130V. Band intensities were quantified using SigmaGel Scanner.

Sequencing

Purified DNA from RT-PCR product or agarose gel was diluted and quantified using spectrophotometer. 80ng of the purified DNA was mixed with 1ul of 10 pmol/ul primer and ddH_2O to 12ul. The samples were then sent out for sequencing to Genewiz.

Results

AURKA

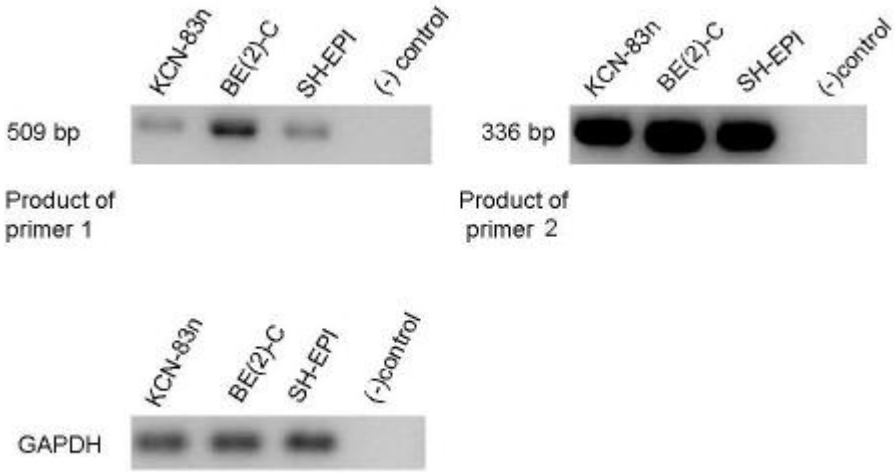

Figure 1-RT-PCR analysis of AURKA transcript levels in N,I and S cells. RT-PCR products generated from RNA isolated from each of the cell lines, amplified by primers recognizing GAPDH and AURKA respectively.

AURKB

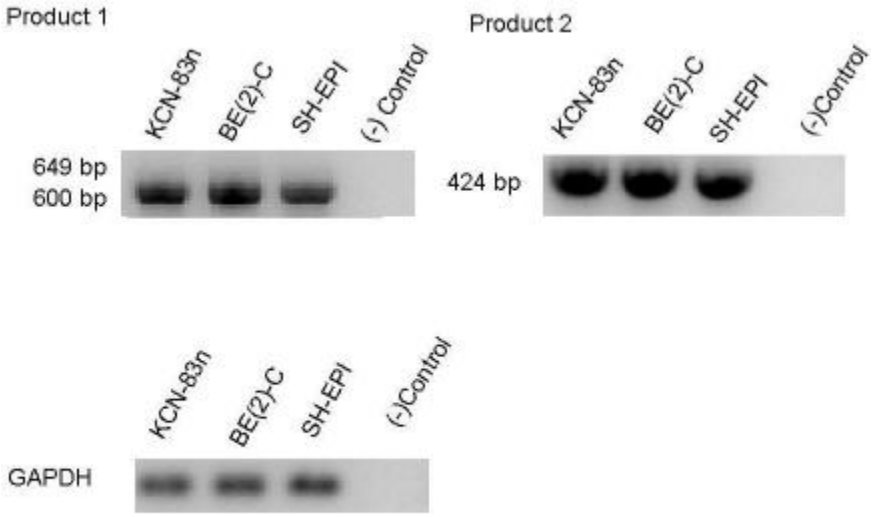

Figure 2-RT-PCR analysis of AURKB transcript levels in N, I and S cells. RT-PCR products generated from RNA isolated from each of the cell lines, amplified by primers recognizing GAPDH and AURKB respectively.

AURKC

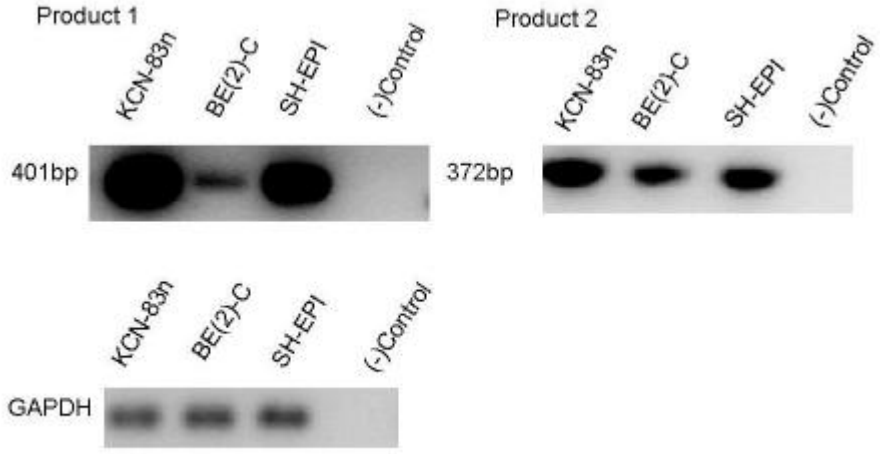

Figure 3-RT-PCR analysis of AURKC transcript levels in N,I and S cells. RT-PCR products generated from RNA isolated from each of the cell lines, amplified by primers recognizing GAPDH and AURKC respectively.

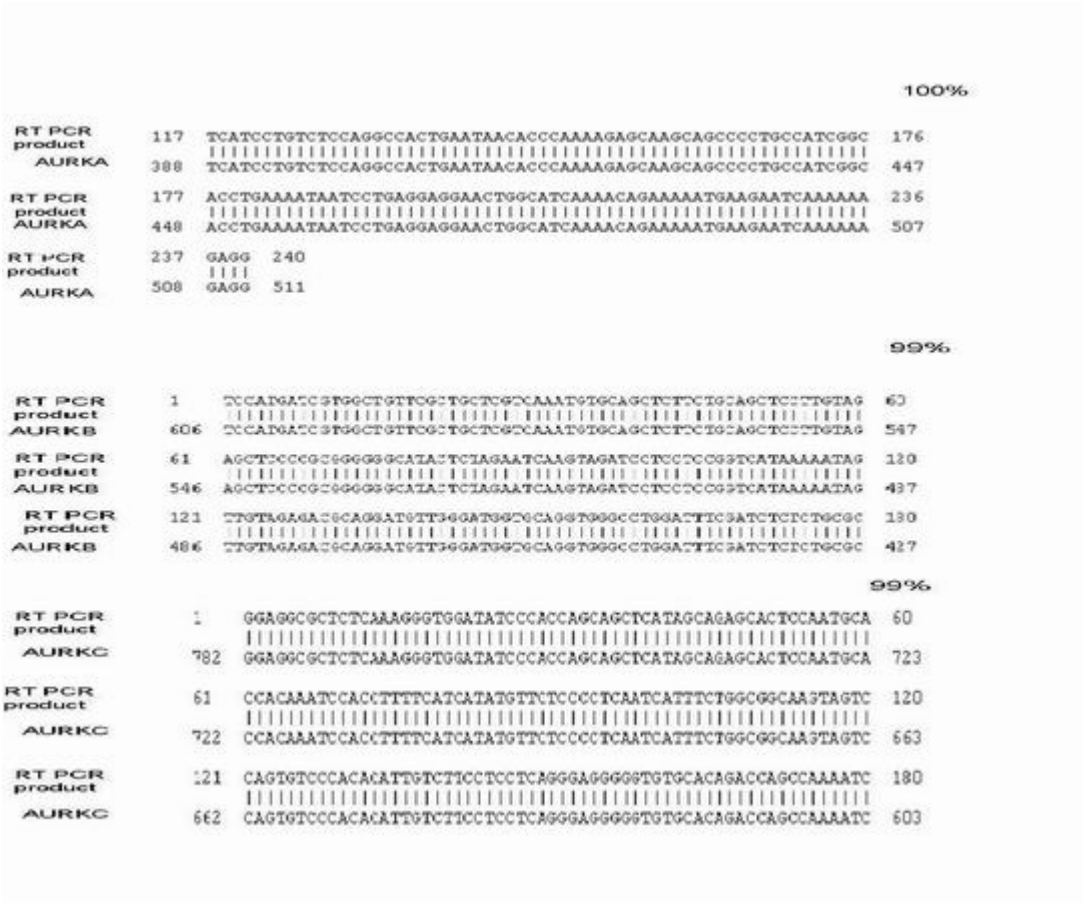

Figure 4. The sequence of the AURKA, AURKB and AURKC amplified using specific primers with a segment of Homo sapiens mRNA sequence from NCBI.

The expression of the AURKA, AURKB and AURKC genes was studied via RT-PCR in three different neuroblastoma cell lines, KCN-83n,SH-EP1 and BE(2)-C. The cycle number was optimized to 38 to see the differential expression AURKA, AURKB and AURKC genes in the above cell lines. Twenty nanograms of Total RNA from each cell line was used for RT-PCR and the products were analyzed on a 1% agarose gel. As an internal control to normalize the variations of RNA quantities within the samples, GAPDH was amplified from equal amounts of RNA in the two cell lines by RT-PCR using GAPDH primers as described in the materials and methods. The RT-PCR products generated using primers for the AURKA, AURKB and AURKC genes were from the respective mRNAs and not from genomic DNA as amplification from the genomic DNA would have generated a much larger size bands (refer to table 1). AURKB is expressed in all 3 cell lines tested and there was no difference in gene expression for AURKB between KCN-83n ,BE(2)-C and SH-EP1 neuroblastoma cell lines (Fig.2). The mRNA for AURKA genes was expressed at a higher level in BE(2)-C ;While the mRNA for AURKB genes was expressed at a lower level in BE(2)-C neuroblastoma cell line (Fig. 1and 3). The partial sequence of each of the three aurora kinase products from RT-PCR amplified using primers specific to each kinase gene, confirmed that

these primers amplified the desired aurora kinase transcript, respectively .(Figure 4)

Discussion

The aim of this project was to investigate the mRNA expression levels of the aurora kinase genes in N,I and S neuroblastoma cells. The RT-PCR analysis suggested a higher expression for AURKA mRNA in I, while Aurora C is expressed the lowest in the I cell type, as compared to N and S cells. There appeared to be no significant difference in AURKB mRNA expression in the three cell phenotypes.

Consistent with results from RT-PCR of this study, several other investigators reported increased expression of AURKA mRNA in highly invasive cancer cells.The reason of decreased AURKC expression in I cells might show that AURKC have opposite function in tumorigenesis .(Katayama H, Brinkley WR, Sen S (2004). "The Aurora kinases: role in cell transformation and tumorigenesis.". Cancer Metastasis Rev. 22 (4): 451–64.)

Based on the obtained data, it cannot be concluded that, the increased expression of AURKA and decreased expression of AURKC have direct relation of the invasiveness of neuroblastoma cell. There are other factors that may also affect the invasiveness of these cells. Due to the increasing relevance of abberations in the aurora kinase dependent pathway in human tumorigenesis, further studies are warranted to study the level and activity of other components of this pathway . Research in this direction could prove useful to develop molecular therapeutics targeting Aurora Kinases.

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