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PCNP is a Novel Regulator of Neuroblastomas Cancer

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Abstract

Estimates of the worldwide incidence and mortality from 27 major cancers and for all cancers combined for 2012 are now available in the GLOBOCAN series of the International Agency for Research on Cancer. Neuroblastoma is a highly heterogeneous tumor accounting for 15% of all pediatric cancer deaths. Neuroblastoma is an embryonal malignancy pediatric tumor that from sympathetic neural crest cells and showing heterogeneous biological, morphological, genetic and clinical behaviour, ranging from fatal progression to differentiation into benign ganglioneuroma. The correct stratification of neuroblastoma patients within risk groups (low, intermediate, high and ultra-high) is critical for the adequate treatment of the patients. Neuroblastoma remains a significant challenge as high-risk. Patients are treated with intensive multimodal therapies but cure rates remain suboptimal. Even targeted chemotherapies against solid cancers show a moderate success increasing the need to novel targeting strategies. To address this problem we review the effect of protein on neuroblastoma. PEST-containing nuclear protein (PCNP), a novel nuclear protein, is involved in cell proliferation and tumorigenesis. However, the precise mechanism of action of PCNP in the process of tumor growth has not yet been fully elucidated. PCNP successfully mediates the proliferation, migration, and invasion of human neuroblastoma cells through mitogen-activated protein kinase and PI3K/AKT/mTOR signaling pathways, implying that PCNP is a therapeutic target for patients with neuroblastoma.

Keywords: PEST-containing nuclear protein; Neuroblastoma; Canada; Role in cancer

Introduction

Globally, one in eight deaths is caused by cancer [1] which includes 100 plus different disorders, originated from many human body cells and organs with various causes and epidemiology. Cancer is characterized by uncontrolled multiplying cells that can percolate distant organs and attack beyond normal tissue boundaries. David von Hansemann [2] and Theodor Boveri [3] in the late nineteenth and early twentieth centuries first studied the crucial part of genome in the development of cancer. They observed strange chromosomal aberrations in dividing cancer cells under the microscope and stated that the aberrations in genetic material cause and characterize irregular clones of cells called cancers. The statement was supported after discovering DNA as the molecular unit of heredity [4] and determining its structure [5] by the demonstration that cancer may also be caused by the substances, damaging DNA and producing mutations in it [6]. Resultantly, an advancement in the analyses of cancerous cell chromosomes revealed that a specific type of cancer is accompanied by particular and repeated anomalies in genetic material like in chronic myeloid leukaemia (known as the 'Philadelphia' translocation) [7,8], translocation occurs between chromosomes 9 and 22.

In conclusion, it was established that phenotypically normal NIH3T3 cells could be converted into cancer cells [9,10] if total genomic DNA from human cancerous cells is introduced into them. This transforming activity was exhibited by a particular segment of DNA which was identified as the first naturally occurring change in human cancer-causing sequence. The change was the replacement of single nitrogenous base of G by T causing the substitution of glycine by valine in 12th codon of HRAS gene [11,12]. This important finding in 1982 initiated an era of dynamic research of the abnormal genes responsible for the human cancer development that lasts till now.

Neuroblastoma

All over the world, children of age less than a year, are the most common victims of neuroblastoma which comprises about 28% of all cancers diagnosed in US and European infants. About 700 cases of neuroblastoma in USA and Canada, and 1500 cases in Europe arise every year [13-16]. Neuroblastoma may be diagnosed in either childhood or parenthood but occurs mostly during infancy, with most cases happening in early childhood. Its incidence falls by half in the second year of life. For childhood tumours, a small number of risk factors have been known in comparison with cancers occurring in adulthood that have made neuroblastoma a challenge. This is the reason that the actual cause of neuroblastoma is poorly understood. Neuroblastoma is a malignant tumor of the neural crest cells which are formed in the 3rd to 4th week of embryonic development. Some of the neural crest cells undergo differentiation and migrate to form the sympathetic nervous system [17].

Neuroblastoma occurs most often in the adrenal glands (approximately 40%) or somewhere else in the chest, abdomen or pelvis. However, it can be found anywhere along the sympathetic nervous system [14]. International Neuroblastoma Pathology Classification system has classified neuroblastoma on the basis of age, rate of mitosis, grade, presence of calcification for diagnosis and mitosis karyorrhexis index [18]. Currently, several countries including Germany, Japan and Canada have employed screening programs which were related to a vast increase in the occurrence of stage 1 disease. However, a decrease

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in the incidence of late-stage disease was not evident. Researchers found these programs less worthy for neuroblastoma screening as the programs had little or no association with decrease in mortality [19-22]. Currently, a notable increase in the prevalence of neuroblastoma has been found. It is interesting to find if this increase is due to increase or change in the distribution of possible hazards or because of surveillance and screening [23,24]. The causes of neuroblastoma have not yet been fully known. Also, the measures to prevent the disease are not currently present. This review has the objectives of evaluating the present literature on neuroblastoma aetiology and suggesting future epidemiological research with a major focus on children of age <15 years, as neuroblastoma rarely occurs in adulthood.

Descriptive Epidemiology

As already mentioned, neuroblastoma majorly occurs during infancy and early childhood. In a review of 3059 cases in North America, 40% infants, 89% children of age 5 and 98% of age 10 were diagnosed for neuroblastoma [25]. In most countries, the rate of prevailing neuroblastoma is equivalent or slightly higher in boys than girls [13,15,23,26-28]. No differences in disease rate are evident across ethnic groups, though in USA, the rate of disease occurrence for blacks (9.6) or Hispanics (9.9) has been reported lower than for whites (12.8 per million). The reason for the given difference may be a greater medical surveillance in white populations [23]. In UK, where neuroblastoma is less prevalent than other European nations, no evidence for variations in disease incidence between Asians and whites has been reported [29]. In countries like Japan (12.5 per million), Western Europe (12.0), Israeli Jews (15.1), New Zealand (11.9 among the non- Maori population), Canada (11.4), Australia (9.9), USA (9.1) and Cuba (8.9), where medical surveillance for neuroblastoma is greater, the incidence of the disease is higher [15,23] while low and medium resource countries of Africa, Asia (with the exception of Japan), Eastern Europe and Central and South America like Egypt, Zimbabwe, Costa Rica, Uruguay, Thailand, Bulgaria and Poland have low rates of neuroblastoma of 5.4, 4.0, 4.5, 2.9, 2.7, 5.0 and 5.3 per million, respectively in children [23,27].

Survival

Neuroblastoma has long been known a mystery of cancers because of its inconsistent outcomes. Clinically, it is characterized by three different patterns: rapid development to lethal disorder, maturation to benign ganglioneuroma and unprompted lapse. Age of the patient, the stage at which the disease occurs and biological characteristics of the disease determine the probability of patient's survival. The worst scenario is found in children, diagnosed at an age of >15 months. Patients diagnosed either at later stages of disease or with certain molecular biological markers like myelocytomatosis viral associated oncogene, acquire amplification of neuroblastoma derived (MYCN), which happens in infants up to 1 year by 5-10% and in childhood and adolescent cases by 20-30% [30-33]. Categorization of tumor hazards on the basis of stage of the tumor, child age and biology and histology of the tumor define the treatment strategies. Generally, a modest improvement in survival has occurred in recent years. 58% survival rates in the USA and Europe and lower in countries of Eastern Europe have been reported in 5 years [15,34-36]. Instead, the disease has the highest rate of spontaneous regression of any cancer. Post-mortem reviews and case reports of children who had died for other reasons have first described this phenomenon [37]. Over 90% survival rates have been estimated in stage 4 patients without MYCN amplification [38]. Difference in survival rates all over the world depends partly on treatment success, also, the rate of diagnosing asymptomatic cases in infants that would spontaneously regress reflects the difference; some countries have a higher rate of this diagnostics [39].

PCNP

PCNP (PEST proteolytic signal containing nuclear protein) is a novel nuclear protein containing 178 amino acids. It has been identified in the nucleus through database mining and is found to have a role in the regulation of cell cycle and formation of tumor. The PEST motif in PCNP is a peptide sequence containing glutamic acid (E), proline (P), threonine (T) and serine (S) as its main amino acids [40-42]. It is known for the degradation of target proteins by functioning as a proteolytic signal through calpain proteolysis or proteasome pathway [41,43,44]. It is an unstructured region in various protein sequences where it serves as a phosphodegron for recruiting F-box which has ubiquitin E3 ligases that cause degradation and ubiquitination [45,46].

PCNP, after being translated, is ubiquitinated by NIRF (Np95/ICBP90-like RING finger protein), a nuclear protein with an ubiquitin-like domain, YDG/SRA domain, a PHD finger and a RING finger. A gene mapped on a human chromosome 3q12.3 encodes PCNP which exists in three isoforms produced by alternative splicing [39,40]. Interaction of PCNP with NIRF results in modulating the transcriptional activity of NIRF [47]. As indicated by recent studies, the cell proliferation and tumerigenesis may also involve PCNP [47,48]. Nevertheless, the regulatory mechanism underlying the processes of proliferation, migration and invasion of cancer cells has not yet been completely explained.

Alternate or other Names/Synonyms or Alternative name of PCNP

DKFZp781124156, PCNP, PEST proteolytic signal containing nuclear protein, PEST proteolytic signal- containing nuclear protein, PEST-containing nuclear protein [45].

Size of PCNP

Size: 178 amino acids

Molecular mass: 18925 Da

Sequence caution

Sequence=EAW79790.1; Type=Erroneous initiation; Note=Translation N-terminally shortened; Evidence={ECO:0000305};

Sequence=EAW79792.1; Type=Erroneous initiation; Note=Translation N-terminally shortened; Evidence={ECO:0000305} [46].

PCNP protein sequence

MADGKAGDEKPEKSQRAGAAGGPEEEAEKPVKTKT-VSSSNGGESSSRSAEKRSAEEEAADLPTKPTKISKFGFAIGSQTTK-KASAISIKLGSSKPKETVPTLAPKTLSVAAAFNEDEDSEPEEMP-PEAKMRMKNIGRDTPTPNSFNKGKHGFSDNQKLWERNIKSHL-GNVHDQDN.

PNCP origin of localization

PEST-containing nuclear protein (PCNP) is a new type of finger protein. This protein ligase is primarily located in the nucleus. It has an ability of ubiquitination and may cause protein degradation through ubiquitination pathway [47]. It is shown by the studies that cell-cycle regulation may also involve PCNP. However the investigation of the relationship between regulator molecules like PCNP, TIPE2 and RA is seldom (Figure 1) [48].

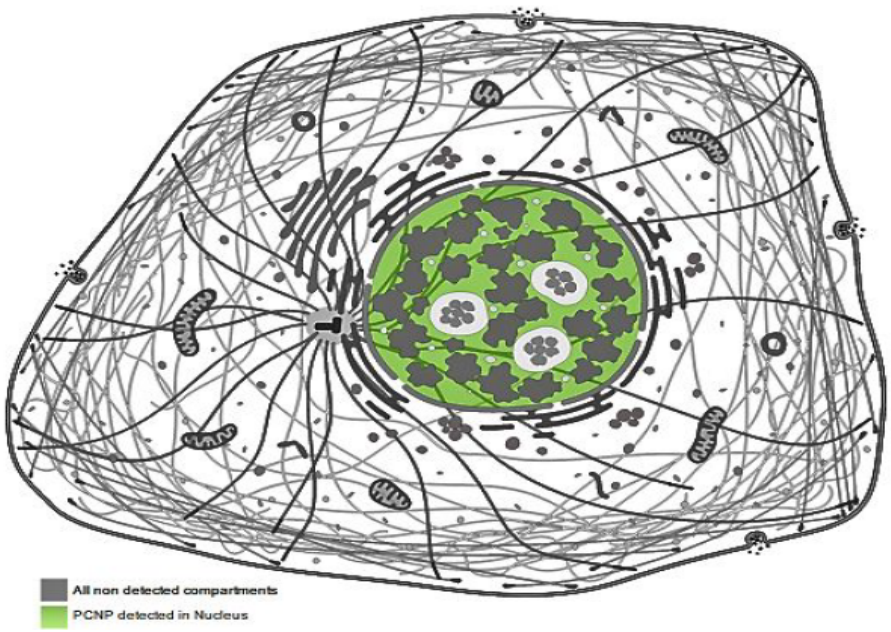

Figure 1: Presence of PCNP in cell.

Genomic locations for PCNP gene

Genomic Locations for PCNP Gene (Figure 2)

chr3:101,573,534-101,594,437 (GRCh38/hg38)

Size: 20,904 bases

Orientation: Plus strand

chr3:101,292,939-101,313,281 (GRCh37/hg19)

Genomic view for PCNP gene

Genes around PCNP on UCSC Golden Path with Gene Cards custom track.

Cytogenetic band

•3q12.3 by Ensembl

•3q12.3 by Entrez Gene

•3q12.3 by HGNC (Figure 3)

Bands according to Ensembl, locations according to GeneLoc (and/or Entrez Gene and/or Ensembl if different) [46].

Exon structure for PCNP

From Entrez Gene (Table 1) [47].

From Ensembl (Table 2) [47].

Conserve sequences of PCNP

Following image shows a graphical summary of conserved domains identified in the query sequence of PCNP. Domains are colour coded according to superfamilies to which they have been assigned [48].

Role of PCNP in Neuroblastoma Cancer

The structure and therapeutic disorders associated with PEST containing proteolytic signal protein have not still studied by the science of proteomics. Based on few data research, PCNP is found to be an important part of cell proliferation [49-51]. The pioneer of PCNP predicted its key role in progression of cell cycle and genome stability, the two processes which are regulated by an intricate system of signaling molecules including ubiquitin ligases, transcription factors, protein kinases and substrate [52-55]. This complex network performs the role of ubiquitin and regulates a signaling pathway for cell homeostasis. This complex system of cellular proteins requires great effort to evaluate the real part of NIRF and PCNP. To study the actual process of cell proliferation, an insight into this novel protein is necessary. Cells need proper signaling cascade to carry out normal functions and these short lived proteins perform a key part in this cascade.

The cells which control proteolysis, majorly involve PEST proteins whose functions are monitored by the mechanisms promoted by the ubiquitin proteasome system [56-59]. Numerous properties of neuronal cells are possessed by the human neuroblastoma cell lines SK-N-SH and SH-SY5Y which have been extensively used as cellular models for evaluating the intracellular mechanisms of therapeutic agents [60-66]. Both in vitro and in vivo effects of PCNP have been investigated in SK-N-SH and SHSY5Y cells by Wu et al. who revealed that PCNP can be expressed in human neuroblastoma cells, besides hepatoma cells, fibrosarcoma cells and myeloid leukemia cells [55]. It was observed that overexpression of PCNP reduced the proliferation and viability of SK-N-SH and SHSY5Y cells, in addition to decreasing their migration and invasion capabilities. While suppression of PCNP displayed completely reverse effects, signifying an important role of PCNP in the growth, migration, and invasion of human neuroblastoma cells.

In multicellular organisms, the normal development and maintenance of tissue homeostasis depends upon programmed cell death or apoptosis [67] which comprises two signalling pathways: a pathway occurring through mitochondria (an intrinsic pathway) and a pathway initiated by death receptors (an extrinsic pathway) [68]. In mammals, the process of apoptosis could be regulated by the dominant proteins of BCl-2 family such as Bax, BCl-2, Bad, and BCl-xl [69].

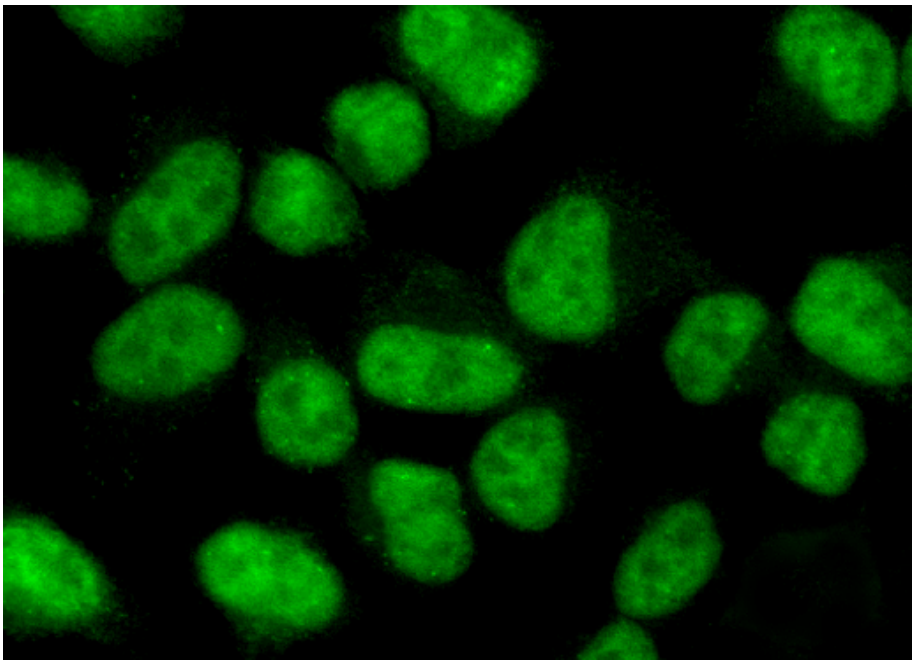

Figure 2: PCNP (K-16). Immunofluorescence staining of methanol-fixed HeLa cells showing nuclear localization.

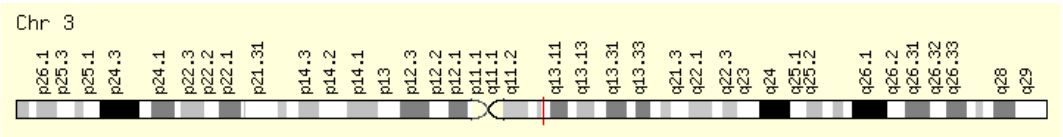

Figure 3: PCNP Gene in genomic location.

Id	Chromosome	Strand	Exon Start	Exon End
57092	3	+	101573534	101574152
57092	3	+	101574153	101574279
57092	3	+	101574198	101574215
57092	3	+	101574216	101574279
57092	3	+	101579790	101580004
57092	3	+	101585437	101585511
57092	3	+	101590215	101590270
57092	3	+	101592627	101592753
57092	3	+	101592754	101594437

Table 1: Entrez Gene.

The apoptotic stimuli activate caspases, resulting in their cleavage of which in turn inactivate PARP to create an apoptotic cascade [70]. This apoptotic index, protein expressions of cleaved caspase-3, 8, 9 and Bad/BCl-xl and Bax/BCl-2 ratios were shown to increase remarkably by PCNP over-expression in Wu et al. studies, signifying the stimulation of mitochondria mediated pathway. On the other hand, the level of apoptosis was found to decrease dramatically as a result of PCNP knockdown, representing the pro-apoptotic function of PCNP in neuroblastoma.

Various functions of a cell including differentiation, proliferation and apoptosis are regulated by MAPKs. The three main subfamilies of MAPK, namely p38, ERK ½ and JNK have been identified [59-61]. It has been found that an increased expression of p-ERK occurs in many cancers, which may result in proliferation of cancer cells and cancer progression [71]. However, in many studies, the apoptosis process has been shown to increase in SH-SY5Y cells by increased p-ERK [72-74]. These contradictions may result from differences in cell lines [75]. Also, the phosphorylation of p38 and JNK can be occurred in rotenone-induced apoptosis in SH-SY5Y cells [76]. Wu et al. showed that the apoptosis which results from over-expression of PCNP occurred by stimulating the phosphorylations of JNK (Thr183/Tyr185), p38 (Thr180/Tyr182) and ERK1/2 (Thr202/Tyr204) in both SK-NSH and SH-SY5Y cells. Though, decreased phosphorylations of JNK (Thr183/Tyr185), p38 (Thr180/Tyr182) and ERK1/2 (Thr202/Tyr204), resulted from PCNP knockdown could promote the growth, migration and invasion of neuroblastoma cells. These findings recommend that the growth process of human neuroblastoma cells can be regulated by PCNP through MAPK signaling pathway.

The PI3K/Akt/mTOR signalling pathway has an important role in stimulating growth, survival, motility and protein synthesis of the cell [62,77,78]. By using a regulators cascade, the serine/threonine kinase Akt is first activated by PI3K, which then phosphorylates and activates mTOR [77]. This activation of PI3K/AKT/ mTOR pathway results in tumour progression and reduced survival of the patient [79]. It is generally believed that PI3K/AKT/mTOR pathway is an auspicious therapeutic target for treating cancer [62,77,80]. It has been shown by a recent study that the inhibition of PI3K/Akt/mTOR signalling in neuroblastoma cells by alectinib could overwhelm cell proliferation and induce apoptosis [81]. Furthermore, an antitumor ability, exhibited by afatinib could induce apoptosis and block the PI3K/AKT/

Gene Id	Exon Id	Chromosome	Strand	Exon Start	Exon End
ENSG00000081154	ENSE00001837860	3	+	101574095	101574279
ENSG00000081154	ENSE00001866824	3	+	101574186	101574279
ENSG00000081154	ENSE00001883923	3	+	101574190	101574279
ENSG00000081154	ENSE00001829458	3	+	101574194	101574279
ENSG00000081154	ENSE00003760567	3	+	101574203	101574279
ENSG00000081154	ENSE00001819552	3	+	101574219	101574279
ENSG00000081154	ENSE00001829577	3	+	101574841	101574933
ENSG00000081154	ENSE00003765040	3	+	101579589	101579647
ENSG00000081154	ENSE00001948971	3	+	101579589	101580004
ENSG00000081154	ENSE00003620981	3	+	101579790	101580004
ENSG00000081154	ENSE00003588189	3	+	101579790	101580004
ENSG00000081154	ENSE00001844360	3	+	101579842	101580004
ENSG00000081154	ENSE00003671454	3	+	101585437	101585511
ENSG00000081154	ENSE00003515634	3	+	101585437	101585511
ENSG00000081154	ENSE00001903110	3	+	101589749	101590270
ENSG00000081154	ENSE00003682974	3	+	101590215	101590270
ENSG00000081154	ENSE00003666087	3	+	101590215	101590270
ENSG00000081154	ENSE00003588145	3	+	101590215	101590270
ENSG00000081154	ENSE00003188105	3	+	101592627	101594437
ENSG00000081154	ENSE00001907107	3	+	101592627	101592875
ENSG00000081154	ENSE00001867135	3	+	101592627	101592771
ENSG00000081154	ENSE00001832725	3	+	101592627	101592776
ENSG00000081154	ENSE00001846412	3	+	101592627	101593251
ENSG00000081154	ENSE00001857104	3	+	101592627	101594407

Table 2: Ensembl.

mTOR signalling in a neuroblastoma xenograft mouse model [82]. Wu et al. suggested that PCNP-related agents can be applied as anti-cancer drugs, for over-expression of PCNP inhibited phosphorylations of PI3K (Tyr458/ Tyr199), AKT (Ser473) and mTOR (Ser2448), and ultimately induced apoptosis. However, increased phosphorylations of PI3K (Tyr458/Tyr199), AKT (Ser473), and mTOR (Ser2448) by PCNP knockdown promoted the growth, migration, and invasion of neuroblastoma cells. These results show that PI3K/Akt/mTOR signalling pathway is the route through which the growth, migration, and invasion of human neuroblastoma cells can be regulated by PCNP.

It has been indicated by a number of studies that SH-SY5Y and SKN- SH cells have been extensively accepted to create subcutaneous xenograft models [63-65]. Wu et al. used BALB/c nude mice to study the effect of PCNP on the growth of neuroblastoma xenograft tumours and observed that the growth was significantly decreased by the over-expression of PCNP. It was also observed that PCNP suppression remarkably enhanced tumour growth. Nevertheless, higher inhibitory rate of tumour by PCNP was shown in SHSY5Y cells than in SK-N-SH cells, and the reason was found to be the difference of the expression level of GD2 ganglioside between SK-N-SH and SH-SY5Y cells [83,84]. Ki67 is a non-histone nuclear protein which can be found in proliferating cells in all stages of the cell cycle except G0 [85]. Its expression closely relate to the multiplication, invasiveness and clinical outcome of a number of malignant tumours [86]. It is considered as an important marker and has been extensively applied to detect the proliferation of malignant cells [56,85,86]. In accordance with the above findings, a decreased and an increased expression of Ki67 was observed in the PCNP and sh- PCNP group, respectively. The tumour MVD represents the density of CD31, an ideal biomarker for vascular endothelial cells [75,78]. Wu et al. examined that a reduced expression of CD31 happened by PCNP over-expression in neuroblastoma xenograft tumours, whereas suppression of PCNP enhanced the expression of CD31, signifying PCNP a modulator of the growth of human neuroblastoma xenograft tumours by controlling angiogenesis.

An innovative and molecular based research on PCNP will allow the molecular biologist's to identify new analytical markers. In this way, the mystery behind cell proliferation, gene expression, apoptosis and behavior will also be revealed.

Since Real time PCR revealed high level of PCNP in cancerous cell lines, it has been declared that PCNP play an important role in suppressing tumor growth. Therefore, a research with the purpose of using patent PCNP as an analytical marker and investigative remedy for tumors can be proposed. All over the world, the molecular biology laboratories can take this novel protein an exciting call. Refinement in the molecular methods and more believe in scientific services will become true after long term analysis of PCNP. The dream of human welfare will also come true.

Conclusion

PCNP (PEST containing nuclear protein) could possibly be responsible for tumorigenesis. It may play an important part in various cellular pathways such as proliferation, differentiation, growth, metabolism, cellular transition, cell death, regulation of MAPK and mTOR/PI3K /AKT pathways. PCNP regulates the development and blood vessels generation in human neuroblastoma cells. Therefore, PCNP can prove to be a significant biomarker in detection and therapy of human carcinomas. Though the proper course of action of PCNP is not fully understood yet and requires further research to explore its diverse role in human cells.

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Conflict of Interest

We all authors declare that we have no conflict of interest for this article.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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