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Nestin expression in human tumors and tumor cell lines.

Minireview

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The aim of this review is to summarize current knowledge on nestin expression in human tumors and corresponding tumor cell lines. Nestin belongs to class VI of the intermediate filaments and it is expressed primarily in mammalian nervous tissue during embryonic development. In adults, nestin occurs only in a small subset of cells and tissues. This protein has been observed in the subventricular zone of the adult mammalian brain, where neurogenesis is localized. Nestin expression has also been detected in various types of human solid tumors, as well as in the corresponding established cell lines. This article provides an up-to-date overview of tumors in which nestin has been found. Another aim of this review is to summarize recent findings on the intracellular localization of nestin in human tumor cells, especially with regard to the possible correlation between nestin expression and the malignant phenotype of transformed cells. Nestin expression in vascular endothelial cells during angiogenesis is also reviewed. Special attention is paid to the detection of nestin in cancer stem cells because this protein, together with the CD133 surface molecule, is considered to be a possible marker of cancer stem cells, especially in tumors of neuroectodermal origin.

Key words: nestin, intermediate filaments, cytoskeleton, human tumors, angiogenesis, cancer stem cells

Nestin is a protein consisting of 1621 amino acids that usually occurs in two forms: a 220 kDa glycosylated form and a 177 kDa deglycosylated form [1, 2]. In 1985, this protein was discovered in the developing nervous system of mice, and it was classified as a member of the intermediate filament (IF) class VI [3, 4]. The name "nestin" is derived from its location: **ne**ural **st**em cell prote**in**. At present, nestin is considered to be a marker of neural stem and progenitor cells, although its expression in other cell types is under investigation. A high correlation between nestin expression and cell proliferation in the CNS (central nervous system) has been demonstrated many times. Due to its expression in many types of human solid tumors, nestin may serve also as a diagnostic and possibly prognostic marker of tumor malignancy [4, 5].

1. Nestin expression in mammals under physiological conditions

IFs undergo significant remodeling during mammalian embryonic development. Nestin is strongly expressed in proliferating undifferentiated cells in the subventricular zone (SVZ). Levels of nestin decrease during fetus progression, and nestin is gradually replaced by other IF proteins specific for mature cells: by glial fibrillary acidic protein (GFAP) in glial cells and by three types of neurofilaments in neurons. At this stage, cells are going through a transition period that is characterized by co-expression of immature and mature cell state markers [4, 6]. Although nestin is typical for neural stem cells, it has also been detected in other types of cells and tissues during mammalian embryogenesis, e.g., skeletal muscles [4, 7, 8], umbilical cord blood [9], cardiac muscles [10], Sertoli and interstitial testicular cells [11], odontoblasts [12], hair follicle sheath cells [13], hepatic cells [14], and renal progenitors [15].

In adults, new neurons arise from the neural stem cell population expressing nestin in the SVZ and the hippocampus [16, 17, 18, 19]. Strong nestin expression has also been detected in reactive astrocytes during regeneration after injury to the nervous system [20, 21]. Nestin has also been detected in other types of adult mammalian cells and tissues, e.g., in the interstitial cells of Cajal [22, 23], pancreatic islet cells [24], retina

[25], bone marrow stromal cells [26], Leydig cells [2], myocardium [27], oval cells in adult liver [28], renal podocytes [15], adenohypophyseal corticotrops [29], and in the regenerative compartment of the mammary gland [30]. Therefore, nestin expression is not limited to the nervous system and appears in healthy tissue as well as in injuries or other pathological events. [31]. In general, the presence of nestin in the cells of adults indicates an undifferentiated state, plasticity, increased mobility, or pathological conditions [4, 21, 31].

2. Nestin expression in human solid tumors and tumor-derived cell lines

Recently published studies have shown that some cancer cells have many similar features to cells occurring in mammals during embryogenesis. One of these similarities is the expression pattern of IF proteins. Nestin expression has been detected in many types of neurogenic tumors, as well as in tumors of different origins. These results could imply that the mechanisms controlling the expression of the nestin gene in cells of developing organisms are similar to those in transformed cells [32]. Since nestin is the IF protein typical of neuronal and myogenic precursors, one could expect that this protein occurs specifically in tumors arising from these two lineages [22]. However, nestin has been detected in many more tumor types than those mentioned previously.

2.1. Nestin in neuroectodermal neuroepithelial tumors

Neuroepithelial tumors are a type of primary central nervous system tumors. In accordance with their histogenetic origin, nestin was detected in most types of these tumors, usually in both tumor cells and vascular endothelial cells (Table 1). In general, nestin is strongly expressed, especially in tumors that arise from undifferentiated precursor cells and immature progenitors.

In astrocytic tumors, the presence of nestin in the cytoplasm of transformed cells was proved many times. It has been observed that nestin expression correlates with tumor malignancy and invasiveness [33, 34]. In subependymal giant cell astrocytomas, pilocytic astrocytomas, and fibrillary astrocytomas, nestin is expressed weakly. However, in high grade types, such as in anaplastic astrocytoma and glioblastoma multiforme, nestin expression is strong [35, 36, 37]. Ehrmann et al. presumed that the intensive nestin expression in high grade astrocytomas (grade III and IV) probably indicates an immature and invasive phenotype of transformed cells [33]. Similarly, strong nestin expression was also found in tumor cells of anaplastic oligodendrogliomas (grade III-IV), whereas only minimal expression was detected in oligodendrogliomas (grade II) [32, 38, 39]. Furthermore, nestin expression was confirmed in embryonic tumors that arise from undifferentiated precursors in various locations within the nervous system: in medulloblastomas [32, 35, 36], neuroblastomas [40, 41], and retinoblastomas [42].

Table 1: An overview of published findings on nestin detection in neuroectodermal neuroepithelial tumors.

Tumor type (grading)	TCs	VECs	References
ASTROCYTIC TUMORS			
Subependymal giant cell astrocytoma (I)	+	+	33
	+	*na*	34
	+	-	35
Pilocytic astrocytoma (I)	-	+	32
	+	+	33, 36, 39
	-	*na*	34
	+	-	35
	+	*na*	38
Fibrillary astrocytoma (II)	+	+	32
	+	+	33, 39
	-	*na*	38
Anaplastic astrocytoma (III)	+	+	32, 39
	+	+	33
	+	*na*	35, 38, 43
Glioblastoma multiforme (IV)	+	+	32, 33, 35, 39
	+	+	36
	+ / N	*na*	37, 43
	+	*na*	38
Gliomatosis cerebri (III-IV)	+	*na*	45
OLIGODENDROGLIAL TUMORS			
Oligodendroglioma (II)	+	+	32, 39
	-	*na*	38
Anaplastic oligodendroglioma (III)	+	+	32, 39
	+	*na*	35
OLIGOASTROCYTIC TUMORS			
Oligoastrocytoma (II)	+	+	39
Anaplastic oligoastrocytoma (III)	+	+	39
EPENDYMAL TUMORS			
Subependymoma (I)	-	-	34
	+	+	39
Ependymoma (II)	+	+	32, 36, 39
	+	*na*	34, 35, 38
Anaplastic ependymoma (III)	+	+	32, 39
CHOROID PLEXUS TUMORS			
Choroid plexus papilloma	-	-	32
NEURONAL AND MIXED NEURONAL-GLIAL TUMORS			
Dysembryoplastic neuroepithelial tumor (I)	+	*na*	46
Ganglioglioma (I)	-	+	39
	+	*na*	47
Papillary glioneuronal tumor PGNT (I)	+	+	48
Central neurocytoma	-	+	34
	+	*na*	35
EMBRYONAL TUMORS			
Medulloblastoma (IV)	+	+	32
	+	*na*	35
	-	+	39
Neuroblastoma (IV)	-	-	32
	+	*na*	35
	+ / N	+	40
	+	*na*	41
Primitive neuroectodermal tumor (PNET)	+	+	32
	+	+	36
Atypical teratoid / rhabdoid tumor (IV)	-	+	39
Retinoblastoma	+	*na*	42

Nestin expression (+) in the cytoplasm of tumor cells and of vascular endothelial cells is distinguished; N refers to the detected localization of nestin in cell nuclei. TCs, tumor cells; VECs, vascular endothelial cells; *na*, data not available.

Table 2: An overview of published findings on nestin detection in other tumor types.

Tumor type (grading)	TCs	VECs	References
OTHER NEUROECTODERMAL TUMORS			
Melanocytic nevus	-	*na*	33
	+	+	48
	+	*na*	56
Malignant melanoma	+	*na*	33
	+	+	48
	+	*na*	56
Granullar cell tumor (GCT) (I)	+	*na*	51
Phaeochromocytoma	-	*na*	33
Schwannoma (I)	-	*na*	22, 33
	+	+	39
	+	*na*	52
TUMORS OF MENINGOTHELIAL CELLS			
Meningioma (I)	-	+	39
Atypical meningioma (II)	-	+	39
MESENCHYMAL TUMORS			
Angiosarcoma	+ / N	+	53
Rhabdomyosarcoma	+	+	8
	+	-	32
Osteosarcoma	+	*na*	57
Dermatofibrosarcoma protuberans	+	+	58
Dermatofibroma	-	+	58
Gastrointestinal stromal tumor GIST	+	*na*	22, 52, 53
Leiomyoma	-	*na*	22, 52
Capillary haemangioma	-	+	33
Cavernous haemangioma	-	-	33
Hemangioblastoma (I)	*na*	+	39
GERM CELL TUMORS			
Germ cell tumors of CNS			
Germinoma	+	*na*	54
Embryonal carcinoma	+	*na*	54
Choriocarcinoma	+	*na*	54
Yolk sac tumor	+	*na*	54
CNS teratoma	-	*na*	54
Testicular tumors			
Intratubular neoplasia	-	+	2
Teratoma	+	+	2
Seminoma	-	+	2
EPITHELIAL TUMORS			
Pituitary adenoma	-	+	29
Pancreatic adenocarcinoma	+	-	53
	+	*na*	59
Breast tumors	+	*na*	30
Ovarian tumors	+	*na*	60
Apocrine mixed tumor of the skin	+	+	61
Trichoblastoma	-	+	61
Sebaceoma	-	+	61
Anaplastic thyroid carcinoma	+	*na*	62

Nestin expression (+) in the cytoplasm of tumor cells and of vascular endothelial cells are distinguished; N refers to the detected localization of nestin in cell nuclei. TCs, tumor cells; VECs, vascular endothelial cells; *na*, data not available.

A relatively surprising finding was the presence of nestin in the cell nuclei of glioblastoma and neuroblastoma tumor cells [37, 40, 43]; however, its function in the nucleus remains unclear. Thomas and co-workers investigated a possible correlation between N-myc and nestin expressions using neuroblastoma cell lines. They found that the amount of nestin in tumor cells is associated not so much with *MYCN* gene amplification as it was with high levels of N-myc protein. They also observed that low nestin expression in neuroblastoma cells is related to lower malignancy and metastatic potential. If the level of N-myc protein was experimentally reduced, the level of nestin was significantly decreased as well. These results indicate that the presence of N-myc protein and nestin transcription may be related. The second intron of the nestin gene contains 5′CACGTG and 5′CACGAG regulatory elements, and the authors showed that N-myc protein binds to these elements with high affinity, and thus affects transcription of the nestin gene in neuroblastomas. The authors also showed that nestin occurred particularly in the nuclei of cells with *MYCN* amplification, and they proved that nestin interacts directly with the nuclear DNA. Therefore, they hypothesize that nestin may be involved in the regulation of gene expression [40]. On the contrary, Korja and colleagues performed similar experiments on mice and their results show that the amount of nestin is not associated with the N-myc levels in neuroblastoma cells [41].

Using small interfering RNA (siRNA), Wei and co-workers managed to decrease the frequency of nestin positive cells in cultured astrocytoma cells. They concluded that a suppression of tumor cell growth induced by the nestin siRNA may provide a potential novel tool in the treatment of astrogliomas [44].

2.2. Nestin in other tumor types

In addition to its expression in neuroectodermal neuroepithelial tumors, as described in the previous text, nestin was also detected in other types of human solid tumors. In the next part, nestin-positive tumor types occurring outside the CNS are listed (Table 2). Nevertheless, some of them may have intracranial localization under certain conditions. In general, nestin expression both in tumor cells and vascular endothelial cells was detected in various types of neuroectodermal, epithelial, and mesenchymal tumors.

In regard to neuroectodermal tumors of the skin, cytoplasmic nestin has been shown in tumor cells of benign melanocytic nevi as well as of malignant melanoma [49] and its expression correlates with a malignant phenotype of the tumors [33]. Moreover, some nestin-positive populations of tumor cells have a primitive phenotype and this finding supports the cancer stem cell hypothesis (see below) [50, 51]. The cytoplasmic nestin was also found in granular cell tumors (GCT), which are derived from Schwann cells and are mostly benign [52].

Among non-epithelial tumors of the gastrointestinal tract, nestin expression was analyzed in gastrointestinal stromal

tumors (GIST), leiomyomas, and schwannomas. Nestin was detected in GIST and schwannomas, while leiomyomas were nestin-negative [22, 52]. Similarly to astrocytic tumors, a significant difference between the amount of nestin in benign and malignant GISTs has been found [52]. Based on Sarlomo-Rikala´s findings, it is assumed that non-epithelial tumors in the gastrointestinal tract, i.e., gastrointestinal stromal tumors (GIST), schwannomas and GCT arise from multipotent precursor cells expressing nestin [53].

Nestin expression was also confirmed in sarcomas, both of bone and soft tissues (Table 2). A significant difference in nestin expression has been detected in angiosarcomas of various malignant grades. Less-differentiated angiosarcoma grade III cells are similar to their endothelial precursors, for which nestin expression is typical. Yang and co-workers thus hypothesize that angiosarcomas originate from these non-transformed precursor endothelial cells. Furthermore, nestin has also been confirmed in the cell nuclei of some malignant angiosarcomas [54].

Germ cell tumors represent a heterogeneous group of neoplasms. Nestin expression of varying intensity was reported in germinomas, choriocarcinomas, embryonal carcinomas, and yolk sac tumor cells; however, nestin was not detected in matured teratomas. Similar to above, Sakurada and co-workers also showed that nestin levels are related to tumor progression rate, i.e., to the malignancy [55]. Among testicular tumors, transformed cells of carcinoma *in situ* and seminoma were determined to be nestin-negative and nestin was observed only in Sertoli cells nearby the carcinoma *in situ* [2]. In non-seminomas, tumor cells of neuroepithelial and mesenchymal teratoma component showed cytoplasmic nestin, in contrast to other teratoma cells [2]. Therefore, nestin expression in the neuroepithelial and mesenchymal teratoma components could serve as a useful marker of less differentiated neoplastic cells [2, 22].

Nestin expression was also shown in pancreatic adenocarcinoma cells. Nevertheless, a significant difference in nestin levels between low-grade and high-grade adenocarcinomas has not been detected [54].

Kolar and co-workers confirmed the presence of nestin in stromal cells of invasive ductal breast carcinoma. Nestin is detectable in transformed epithelial cells, stromal elements, and in endothelial cells, which form tumor capillaries [56]. Determination of nestin expression can be used to estimate the biological behavior of the tumor because nestin has been detected in the invasive carcinoma subtype, which is less differentiated, more aggressive, and has a poorer prognosis. [30, 56].

3. Nestin expression during angiogenesis

Angiogenesis has great importance in tumor diseases because this process enables tumor growth, as well as formation of metastases. It is already known that highly vascularized tumors usually have a worse prognosis [56, 64, 65]. Surface molecules CD34 and CD31 are considered to be markers of vascular endothelial cells. In contrast, nestin has been detected in proliferating vascular endothelial cells only. For this reason, nestin is regarded as a valuable marker of neovascularization under both physiological and pathological conditions in organisms [66]. Newly formed endothelial precursors were detected as nestin-positive [56, 64] and a correlation between nestin and vascular endothelial growth factors expressions has also been confirmed [65]. Simultaneously, many other authors demonstrated nestin expression in proliferating vascular endothelial cells near the tumors. In addition to its expression in tumor cells, nestin was detected in vascular endothelial cells in all types belonging to the neuroectodermal neuroepithelial tumors (Table 1), in other neuroectodermal tumors, in mesenchymal as well epithelial tumors, and in germ cell tumors of CNS (Table 2). Some results are in accordance with the hypothesis that vascular endothelial cells represent a part of the CSCs (cancer stem cells) niche (see below) [67]. Therefore, nestin-positive cells representing newly formed endothelium as well as potential CSCs could play both direct and indirect roles in tumor formation and progression.

4. Nestin as a marker of cancer stem cells

During past few years, cancer is considered to be a disease caused by disorders of normal, non-transformed stem cells (SCs) in the respective tissue or organ. According the CSCs hypothesis, tumors – similar to normal adult tissue - contain a small population of transformed SCs. These cells are termed as cancer stem cells or tumor-initiating cells (TICs) [51]. Regulation pathways typical for normal SCs (Notch, Wnt, Sonic hedgehog) have also been found in certain types of CSCs. In addition, many molecules expressed by normal SCs are also detectable in their malignant counterparts. These molecules are probably involved in the maintaining of "stemness", ensuring adhesion to the niche, and in cytoprotection [68]. This minor tumor component (approximately 0.5 - 5% of all cells in the tumor) is responsible for the differentiation of other tumor cells, and consequently for tumor growth and progression of the disease. CSCs are also able to form the same tumor type after implantation into immunodeficient mice [51, 69, 70]. Although the first evidence concerning CSCs was found in leukemia, these cells have been demonstrated in many solid tumors [70]. Another important feature of CSCs is their resistance to conventional chemotherapy and radiotherapy, because these therapies affect more differentiated and proliferating tumor cells only, not CSCs directly [69, 71]. Goodell and co-workers described a method for the isolation of hSCs (human stem cells) based on their ability to efflux a fluorescent dye [72]. If the cells are subjected to staining by Hoechst 33342 and subsequent analysis using fluorescence activated cell sorter (FACS), cells that actively efflux the Hoechst 33342 appear to be a distinct cell population; hence it was termed a "side population" (SP) [70, 72]. The existence of the "side population" has been confirmed both in adult SCs and CSCs

[73, 74]. CSCs have been found in acute myeloid leukemia [51, 75], multiple myeloma [76], as well as in solid tumors of breast [77], CNS [42, 78], prostate [79], pancreas [80], head and neck [81], intestine [82], and in melanomas [83].

At present, nestin and the cell surface molecule CD133 are considered to be characteristic markers of neural SCs. Subpopulations of nestin positive cells were identified as neural SCs using nestin/EGFP transgenic mice generating neurospheres [82], but the presence of nestin was shown in the progenitor cells as well [83]. In brain tumors, clonogenicity (i.e., neurosphere forming) and self-renewal are exclusive to a minor subpopulation of tumor cells expressing CD133 and nestin. Therefore, these CD133/nestin positive cells are recognized to be a potential CSC population, but the mechanism of stem cell transformation still remains unclear [69, 78, 86]. These cells had not only the capacity to differentiate into cells with neural and glial phenotypes *in vitro* in proportions resembling the original tumor, but also their proliferative capacity was proportional to the aggressiveness of the original tumor [70]. Calabrese and colleagues have demonstrated that CD133/nestin positive CSCs are localized in close proximity to the vascular endothelial cells in certain brain tumors and they may directly interact with the vascular endothelial cells. They revealed that vascular endothelial cells secrete factors that give CSCs a self-renewal potential and that maintain them in an undifferentiated state; thus, the vascular endothelial cells form the CSCs niche. An experimental reduction or increase in the number of vascular endothelial cells suppressed or supported the emergence of CD133/nestin positive cells and the tumor growth. These finding suggested that the ability to stimulate the formation of blood vessels in the surrounding tissues is closely related to the tumor aggressiveness [68]. In addition to the tumors of neurogenic origin, coexpression of nestin and CD133 was recently also confirmed in osteosarcomas [58].

5. Conclusion

Since nestin expression was demonstrated in cell types other than those arising from the neuroepithelial lineage, it cannot be regarded as an exclusive marker of neural stem cells. The presence of nestin in cells points to the primitive phenotype or pathological condition of these cells. Nevertheless, the regulation of nestin expression in undifferentiated cells and its replacement by GFAP or neurofilaments in mature cells is not known. Mechanisms regulating nestin expression in various solid tumors also remain unclear. It was confirmed repeatedly that the nestin levels in tumor cells correlate with malignant grade and undifferentiated state of the tumor. This suggests that nestin expression could be related to a failure of certain key regulatory pathways in the stem cells, especially in neurogenic tumors. Nestin localization in cell nuclei as described in some tumor types has still not been explained. Further detailed research of nestin in solid tumors is required to help answer some of these questions.

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