Review paper

Emerging biomarker in carcinogenesis. Focus on Nestin

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Adv Dermatol Allergol 2022; XXXIX (6): 1001–1007
DOI: https://doi.org/10.5114/ada.2022.122599

Abstract

Nestin is a protein belonging to class VI intermediate filaments, which is involved in organogenesis, cellular metabolism and cytoskeletal organisation. Originally found to be expressed in neuroepithelial stem cells, nestin is also expressed in other tissues. It plays an important role in the carcinogenesis and angiogenesis. Its increased expression in melanoma is associated with an aggressive course of the disease and poor prognosis. Research findings for non-melanocytic skin neoplasms are inconclusive. The aim of this paper was to systematize knowledge on the role of nestin in cancerogenesis. The authors focused in particular on the expression of nestin in skin malignancies, as well as on the potential role of nestin in the pathogenesis, prognosis and treatment of cutaneous neoplasms.

Key words: nestin, carcinogenesis, angiogenesis, basal cell carcinoma, squamous cell carcinoma, melanoma, cancer.

Introduction

A recently discovered protein, nestin, was originally found to be expressed in neuroepithelial stem cells. Since then further studies have shown that nestin is also expressed in other tissues, including skeletal muscle satellite cells, developing myotomes, testes, pancreatic islets, hair follicles, heart, bone marrow and the skin [1–7] and it has emerged as an interesting new marker of physiological and pathological processes.

In this review, we present the current understanding of the role of nestin in carcinogenesis, with a special focus on the skin. We have also systematized the available world literature on the expression pattern and potential role of nestin in the pathogenesis, prognosis and treatment of cutaneous neoplasms.

The impact of nestin on the processes involved in carcinogenesis

Nestin is a protein belonging to class VI intermediate filaments, which is involved in organogenesis, cellular metabolism and cytoskeletal organization. It consists of 1621 amino acids with an estimated molecular weight of 177.4 kDa. Like other intermediate filaments, nestin plays an important role in maintaining the structural integrity of cells and tissues [8]. It is also presently known to be involved in various cellular processes, including cell migration, proliferation and apoptosis [9, 10]. Many studies have suggested an important role of nestin in carcinogenesis. Research on murine models using Huh7 hepatocellular carcinoma and A172 glioblastoma cell lines have shown that nestin silencing reduced *in vivo* tumorigenesis [11, 12].

The signalling pathway of phosphatidylinositol-3-kinase and Akt protein kinase is one of the main systems activated during carcinogenesis, responsible for modulating cellular processes, such as proliferation and migration [13, 14]. Dysregulation and overactivation of the PI3K-Akt-mTOR pathway have been shown to play an important role in tumorigenesis. Reduced expression of nestin has been linked to diminished phosphorylation of key proteins, including Akt and GSK3 [15].

The effects of nestin on tumour cell proliferation was investigated in non-small cell lung cancer in *in vitro* studies. Nestin silencing in cancer cell lines A549 and H460 limited colony forming and the rate of proliferation, which is expressed as a decrease in viable cells, was reduced in nestin-downregulated cells. Furthermore, nestin-downregulated cells were also characterised by diminished expression of Ki-67 proliferation marker. Additionally, DNA synthesis in nestin-downregulated cells was significantly inhibited compared to cancer cell controls [15].

The ability to increase carcinogenesis by inhibiting suppressor genes is another important mechanism of action of nestin. In hepatocellular carcinoma nestin expres-

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Received: 6.07.2021, **accepted:** 13.09.2021.

sion correlated with low expression of the p53 protein, which was also associated with a low survival rate [11].

Cellular resistance to apoptosis is an important defence mechanism of cancer cells, allowing them to survive, among other things, chemotherapy. It was shown in glioblastoma that a population of nestin-expressing stem cells correlated with chemo-resistance [16]. Nestin has been also shown to have an impact on the AKT/mTOR signalling pathway, which plays a key role in autophagy – another mechanism of resistance to cell death [17, 18].

Nestin as a marker of angiogenesis

Angiogenesis is a complex process by which new capillaries are formed by extending and branching the network of already existing vessels, which is stimulated by pro-angiogenic factors [19]. It can be a part of a physiological process, such as embryogenesis, or pathological processes, including carcinogenesis. Vascularization is modulated by a complex of stimulatory and inhibitory factors. The degree of angiogenesis is assessed based on the microcapillary density index (MVD), using antibodies against CD34, CD31 and factor VIII. In pancreatic cancer, expression of CD34, CD31 and factor VIII was observed in both newly formed small vessels and large existing vessels, while nestin was expressed only in new vessels. Therefore, it seems that nestin can be used as a reliable marker of neoangiogenesis. It was also observed that inhibiting nestin expression using a gene-silencing technique inhibited the growth of vascular endothelial cell lines. Therefore, nestin may be a target for inhibiting angiogenesis in pancreatic cancer [20].

The prognostic value of nestin and its impact on angiogenesis was assessed in ductal breast cancer cells. An immunohistochemical evaluation of 124 ductal carcinomas was performed with the use of monoclonal antibodies targeting nestin, CD31, CD34, SOX-18 and Ki-67. A correlation was shown between nestin expression in tumour cells and its expression in vascular endothelial cells. A positive correlation was also found between vessels expressing nestin and SOX-18. The study confirmed previous reports that the expression of nestin in endothelial cells correlates with triple-negative breast cancer, poor (G3) differentiation, higher proliferation index and shorter survival. The expression of nestin was also assessed in cell lines representing different grades of malignancy. More aggressive MDA-MB-231 and BO2 breast cancer cells had higher nestin expression levels than MCF-7 and SK-BR-3 cells. The authors believe that the increased expression of nestin in neoplastic cells is associated with increased angiogenesis; however, the underlying mechanisms require further studies [21].

Many reports have confirmed that nestin may be a reliable marker of proliferating endothelial cells in tissues where neovascularisation occurs [22–24]. However, the role of nestin in the process of angiogenesis remains unclear. The origin of the nestin-expressing vascular endothelial cells is also unknown. A theory has been proposed on the migration of mesenchymal stem cells from the bone marrow [25]. Dong *et al.* demonstrated that human glioblastoma progenitor cells have the ability to transform into vascular endothelial cells *in vitro* and *in vivo* [26]. Similarly, in the skin, hair follicle stem cells show the ability to form new blood vessels [27].

Nestin as a marker of cancer stem cells

There is ample evidence confirming that cancer stem cells (CSCs) play an important role in the process of carcinogenesis. CSCs show a greater capacity for oncogenesis and may be responsible for metastasis [28]. They are detected using specific molecules known as markers.

Due to its potential diagnostic and prognostic value, nestin has been studied in many types of cancer. The first research on the expression of nestin in CSCs was conducted for central nervous system tumours, including medulloblastomas, astrocytomas, ependymomas and gangliogliomas. Nestin was co-expressed with CD133 and SOX-2 markers. A decrease of nestin expression with increasing stem cell differentiation was also observed [29–32].

Nestin expression was also demonstrated in CSCs isolated from mesenchymal tumours, such as rhabdomyosarcoma, osteosarcoma, chondrosarcoma, and fibrosarcoma [33–35].

It has been shown that nestin is a prognostic factor in ovarian cancer [36]. Furthermore, a correlation has been demonstrated between nestin expression and the stage of cancer, lymph node involvement and distant metastases [37]. It was also found that an increased expression of serum nestin in patients with ovarian cancer correlated with a more aggressive course of the disease [38]. An increased expression of nestin in breast cancer cells was shown to be a negative prognostic factor and was associated with the patient's younger age, a higher grade, nodal involvement and shorter survival [21, 39, 40].

The increased expression of nestin also correlated with a poor histological grade, lymph node metastases, TNM classification and tumour size in non-small cell lung cancer, which suggests that nestin can be used as a prognostic factor also in this tumour [41].

Nestin has also been reported to play the role of a marker of cancer stem cells in, among other things, gastric cancer [42], oral squamous cell carcinoma [43], and prostate cancer [44, 45].

Expression of nestin in the skin

The expression patterns of nestin in the skin were described by Sellheyer and Krahl [46]. Nestin is expressed throughout the immature dermis in the early embryogenesis. It is subsequently limited to the follicular connective

tissue sheaths later in the development of hair follicles. Keratinocytes do not express nestin throughout the embryogenesis, while dendritic cells, initially scattered between keratinocytes and then locating in the outer hair sheath, are positive for nestin. Developing blood vessels are nestin-positive throughout embryogenesis. In normal adult skin, nestin is expressed by the cells of the connective-tissue sheaths of hair follicles and perifollicular blood vessels. Fusiform fibroblasts arranged along the connective-tissue sheath and fibroblasts with abundant cytoplasm within the hair papilla are positively stained. Variable nestin expression depending on the hair cycle was also observed, with the strongest expression seen in the early anagen phase. It seems that the mesenchyme-derived connective-tissue sheath (or connective tissue hair follicle) may constitute a niche for mesenchymal skin stem cells. Nestin is considered a marker of mesenchymal stem cells in various tissues.

Nestin expression has been extensively studied in skin cancers such as melanomas, mesenchymal tumours, such as dermatofibrosarcoma protuberans [47, 48] and tumours of the nervous system, such as malignant pe-

Table 1. Literature review of nestin expression in cutaneous tumours

References	Type of skin lesion/ tumour	Nestin expression	Remarks
Flørenes *et al.* [50]	Melanocytic hyperplasia	Positive	Increased expression compared to normal melanocytes
Brychtova *et al.* [51]	Melanoma	Positive	Increased expression of nestin in melanomas compared to benign melanocytic nevi Correlation between the expression level and the severity of the disease
Piras *et al.* [52]	Melanoma	Positive	
Klein *et al.* [53]	Melanoma	Positive	Increased expression in distant metastases
Fusi *et al.* [54]	Melanoma	Positive	Increased number of peripheral nestin-expressing melanocytes in patients with melanoma is associated with poor prognosis
Akiyama *et al.* [55]	Melanoma	Positive	Potential efficacy of nestin-targeted treatment of melanoma
Mori *et al.* [47]	DFSP DF	Positive Negative	Increased expression in DFSP compared to DF
Sellheyer *et al.* [48]	DFSP DF	Positive Negative	
Serra-Guillén *et al.* [56]	DFSP	Positive	Higher expression of nestin is correlated with deeper DFSP invasion
Kanoh *et al.* [60]	Trichoepithelioma SCC BCC	Positive Positive (18.75%) Negative	
Misago *et al.* [61]	Trichoblastoma BCC	Positive Negative	Vascular endothelium within the vascular stroma was positive for nestin in both tumour types
Abbas *et al.* [62]	MCC BCC SCC	Negative Negative Positive (45%)	
Eispert *et al.* [65]	MCC	negative	
Sellheyer *et al.* [66]	BCC Trichoblastoma Trichoepithelioma	Positive Positive Positive	Nestin expression was observed in tumour stromal fibroblasts in the immediate vicinity of epithelial tumour islands in all investigated tumours
Sabet *et al.* [69]	Melanoma BCC SCC	Positive Positive Positive	Nestin expression was higher in melanomas compared to non-melanocytic skin cancers In SCC higher expression was observed in poorly differentiated tumours. In BCC no relationship was found between nestin expression and clinical features or the histological type of the tumour
Lebleci *et al.* [70]	BCC Trichoblastoma	Positive (70.3%) Positive (88.9%)	No statistical difference in the expression of nestin in BCC and trichoblastoma was found

DFSP – dermatofibrosarcoma protuberans, DF – dermatofibroma, SCC – squamous cell carcinoma, BCC – basal cell carcinoma, MCC – Merkel cell carcinoma.

ripheral nerve sheath tumour [49]. Studies assessing the expression of nestin in non-melanocytic skin cancers, such as basal cell carcinoma, squamous cell carcinoma and Merkel carcinoma, are sparse. Table 1 systemizes the current literature on the topic.

Expression of nestin in melanoma

First research on the expression of nestin in melanoma dates back to 1994. It was then noticed that nestin is expressed in melanocytic hyperplasia, while it is not found in normal melanocytes [50]. These findings were confirmed by Brychtova *et al*., who assessed 139 melanoma specimens and compared the expression of nestin in nodular melanomas, superficial spreading melanomas, dysplastic nevi and normal intradermal and dermoepidermal nevi. An immunohistochemical analysis showed an increased expression of nestin in melanomas compared to benign melanocytic nevi, with a correlation between the expression level and the severity of the disease. Nestin overexpression has also been observed in vascular endothelial cells located in the neighbourhood of advanced melanomas [51]. Further studies also showed an increased expression of nestin in the cells located on the periphery of melanoma, responsible for tumour expansion, as well as in the vascular endothelium on the periphery of the tumour [52]. The increased expression of nestin has also been demonstrated in distant metastases of melanoma [53]. It has also been shown that an increased number of peripheral nestin-expressing melanocytes in patients with melanoma is associated with poor prognosis [54]. Akiyama *et al.* used gene silencing to assess the potential efficacy of nestin-targeted treatment of melanoma. They found that a reduced expression of nestin in melanoma cells inhibited cell growth and migration, *in vitro* invasion, as well as *in vivo* tumour growth and metastasis. Therefore, nestin appears to be a promising therapeutic target in the treatment of melanoma [55].

Expression of nestin in mesenchymal tumours

Fibrous tumours are rare, with dermatofibrosarcoma protuberans (DFSP) being the most common one. It is most often found in middle-aged individuals and located in the upper part of the chest. DFSP arises from the fibrous connective tissue. Diagnostic histology uses CD34 and factor XIIIa markers to differentiate DFSP from dermatofibroma (DF). Mori *et al*. investigated the expression of nestin in order to distinguish between these two tumours. Nestin expression was shown in 15/16 DFSP cases (94%) and 4/30 DF cases (13%). These findings support the use of nestin as a marker differentiating between DFSP and DF [47]. Similar results were obtained by Sellheyer *et al.* They reported positivity in all DFSP cases, while all DF samples were negative for nestin [48]. In addition to differential diagnosis, the possible use of nestin in Mohs micrographic surgery was also emphasised. Serra-Guillén *et al.* assessed 71 DFSP cases and found that a higher expression of nestin is also correlated with deeper DFSP invasion [56].

The expression of nestin in skin and adnexal tumours

Skin cancers are the most common cancer group in the Caucasian population worldwide [57], and their incidence has been steadily increasing in recent years. Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), which constitute 99% of NMSCs, are the most common skin cancers. BCC is 3–5 times more common than SCC [58]. Less common skin neoplasms include tumours arising from appendages. Benign tumours of the hair follicle include trichoblastoma, trichoepithelioma, trichoepithelioma desmoplasticum, trichilemmoma, pilomatricoma and trichoadenoma. Like BCC and SCC, these tumours are most commonly found on the face and scalp. Immunohistochemistry with monoclonal antibodies directed against cytokeratin antibodies may be helpful in the differential diagnosis of these neoplasms [59].

In 2008, Kanoh *et al.* investigated the expression of nestin in the epidermis and hair follicles within normal skin and in tumours of epithelial and follicular origin [60]. For this purpose, an assessment of paraffin sections was performed for 11 cases of trichoepithelioma, 16 cases of SCC and 26 cases of BCC. The analysis was also performed on cell cultures. Immunohistochemical staining of normal skin showed no expression of nestin in the basal layer of the epidermis and positive expression in the cells of the hair follicle below the sebaceous glands. In neoplastic lesions, nestin was expressed in all cases of trichilemmoma and in 18.75% of SCCs. No expression of nestin was found in BCC specimens. The authors believe that their results are suggestive of the origin of the tumours. Trichilemmomas appear to arise from the upper part of the hair follicle, below the sebaceous glands, BCCs seem to originate from more mature hair follicle cells in the lower parts of the hair follicle, whereas SCCs from basal epidermal keratinocytes.

In another study, Misago *et al.* assessed the expression of nestin in tumour stromal cells, trichoblastoma (TB) and BCC on paraffin sections. Stromal cells expressed nestin in all trichoblastomas and none of BCCs. However, it was noted that the vascular endothelium within the vascular stroma was positive for nestin in both tumour types. A thesis has been proposed that nestin may be a marker used in the differential diagnosis of these neoplasms and that nestin expression in vascular endothelial cells may indicate its role in neoangiogenesis [61].

In their study, Abbas *et al.* assessed 20 BCC, 20 SCC, and 11 Merkel carcinoma cases for the expression of nestin [62]. Merkel cell carcinoma (neuroendocrine car-

cinoma, MCC) is a rare, aggressive skin cancer. It has a propensity for recurring and metastasizing to lymph nodes and distant organs [63, 64]. In an immunohistochemical evaluation only cytoplasmic staining was considered a positive result. All BCC cases were negative for nestin. The authors believe that nestin does not play a role in the pathogenesis of BCC. In SCC, nestin expression was observed in 9 out of 20 cases (45%). According to the authors, such findings indicate a process of dedifferentiation into stem cells, which is not involved in the process of terminal keratinization. No nestin expression was shown for MCC. Eispert *et al*. also showed nestin-negativity for Merkel cells [65].

Another study by Sellheyer *et al*. [66] compared the expression of nestin in 25 BCCs, 7 trichoblastomas and 11 trichoepitheliomas. In contrast to previous studies, the authors used three anti-nestin antibodies at three different dilutions. It was found that the Atlas polyclonal antibody and the Chemicon monoclonal antibody were more visible compared to the Santa Cruz Biotechnology antibody. Although nestin expression varied depending on dilution, it was evident in all cases. Nestin expression was observed in tumour stromal fibroblasts in the immediate vicinity of epithelial tumour islands in all investigated tumours. Furthermore, the expression of nestin was observed in tumour stromal vessels and within dendritic cells located inside the islands of tumour cells, which were used as positive controls. Using the CD31 marker, it was shown that the reported positive cells in the tumour region are in fact fibroblasts rather than vascular endothelial cells. In the case of BCC, the fibroblasts were fusiform and elongated. In trichoepithelioma and trichoblastoma, the fibroblasts were more spherical and denser, suggesting stronger expression. The authors believe that the above expression pattern in tumours corresponds to the glow pattern in a normal hair follicle. The elongated, fusiform fibroblasts of the hair connective-tissue sheath show similarity to the stromal cells seen in BCC cases, whereas the spherical fibroblasts with abundant cytoplasm seen in trichoepithelioma and trichoblastoma mimic the spherical fibroblasts seen in the hair papilla. The authors believe that adnexal tumours reflect the embryonic development of adnexal structures, but appear to be arrested at different stages of their morphogenesis. Since BCC tends to be regarded as a tumour arising from the cells of the outer hair root sheath [67, 68], the obtained results may support this theory.

In 2014, Sabet *et al*. compared the expression of nestin in 12 melanoma cases, 31 SCCs, and 55 BCCs. All samples tested showed a variable intensity of nestin. A low expression was observed in 30 (55%), moderate in 24 (44%) and strong in 1 BCC (1%). In squamous cell carcinomas, a low expression was shown in 12 (39%) cases, moderate expression in 16 (52%) cases and strong expression in 3 (9%) cases. In the assessed melanomas, a low expression was found in 2 (17%) cases, moderate in 6 (50%) cases and strong in 4 (33%) cases. In SCC cases, the authors observed a higher expression in poorly differentiated tumours. In BCC, no relationship was found between nestin expression and clinical features or the histological type of the tumour. In general, nestin expression was higher in melanomas compared to non-melanocytic skin cancers [69].

Leblebici *et al*. compared the expression of nestin in 27 BCC samples and 27 trichoblastoma samples. Focal expression of nestin was shown in 6 (22.2%) cases of BCC, diffuse expression in 13 (48.1%) cases, and no expression in 8 (29.6%) cases. For trichoblastoma, 7 (25.9%) specimens showed focal expression, 17 (63%) specimens diffuse expression, while no expression was detected in 3 (11.1%) specimens. In this study, no statistical difference in the expression of nestin in these tumours was demonstrated, which contradicts previous reports about the possible use of nestin in the differential diagnosis of the above-mentioned tumours [70].

Conclusions

In the light of recent research, nestin seems to be a new interesting marker of carcinogenesis. However, despite evidence indicating the possible role of nestin in the pathogenesis of skin cancer, the results of the research need to be systematized due to divergences in methodology. It seems that nestin may be a marker used in skin cancer diagnosis, including differential diagnosis. Furthermore, it can be used in clinical practice as a prognostic factor. The expression observed in vascular endothelial cells supports the role of nestin in angiogenesis, which may constitute a starting point for modern targeted therapies. However, the molecular mechanisms underlying these phenomena require further research.

Conflict of interest

The authors declare no conflict of interest.

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