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Review

Nestin expression – a property of multi-lineage progenitor cells?

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Abstract. Tissue-specific progenitor cells are characterized by proliferation and differentiation, but, in contrast to embryonic stem (ES) cells, have limited capacities for self-renewal and no tumourigenic potential. These latter traits make progenitor cells an ideal source for regenerative cell therapies. In this review, we describe what is currently known about nestin, an intermediate filament first identified in neuroepithelial stem cells. During embryogenesis, nestin is expressed in migrating and proliferating cells, whereas in adult tissues, nestin is mainly restricted to areas of regeneration. We show that nestin is abundant in ES-derived progenitor cells that have the potential to develop into neuroectodermal, endodermal and mesodermal lineages. Although it remains unclear what factors regulate in vitro and in vivo expression of nestin, we conclude that nestin represents a characteristic marker of multi-lineage progenitor cells and suggest that its presence in cells may indicate multi-potentiality and regenerative potential.

Key words. Nestin; embryonic stem cells; progenitor cells; differentiation; regeneration.

Introduction

Progenitor cells have the capacity to self-renew and to differentiate into one (unipotent) or several (multipotent) cell types. In contrast to pluripotent embryonic stem (ES) cells, which have an unlimited self-renewal capacity but high tumourigenic potential (for review, see [1, 2]), progenitor cells are defined by high, but limited proliferation and the inability to form tumours. Their restricted self-renewal capacity also prevents clonal growth in vitro [3].
Tissue-restricted progenitor cells have been identified in nearly all somatic lineages, including haematopoietic (for review, see [4]), neural [5], intestinal epithelial [6] or skin epidermal [7] systems. Importantly, these progenitor cells are characterized by the expression of early lineage-specific markers [8]. In adult organisms, tissue-restricted progenitor cells are capable of regenerating tissue-specific cells after injury, but in contrast to adult stem cells, are not capable of long-term regeneration or functional re-establishment of the tissue (for review, see [4, 8]).
The functional properties of both stem and progenitor cells appear to be defined by their natural environment, called the 'niche' [9]. Although, some growth factors and signalling mechanisms regulate the identity of stem and progenitor cells, two of the most active stem cell compartments, the haematopoietic (for review, see [10]) and the intestinal epithelial [11–13] systems appear to be subject to very tight control mechanisms which limit their growth outside the normal niche. In addition to adult stem cells, progenitor cells from different tissues can migrate to and reside in other tissues, i.e. haematopoietic progenitor cells (erythroid, myeloid cells and megakaryocytes)

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in the skeletal muscle [14], bone marrow-derived myogenic progenitor cells in skeletal and cardiac muscle [15] and circulating bone marrow-derived endothelial progenitor cells which home to foci of angiogenesis [16]. This would suggest that not only stem cells [17–19], but also progenitor cells demonstrate plasticity and developmental flexibility. Consistent with this view has been the isolation of multipotent adult progenitor cells (MAPCs) from bone marrow of mouse [20] and human [21]. These cells specifically differentiate into lineages other than the tissue of origin in vitro and in vivo [20, 22, 23]; however, the identity of MAPCs has not yet been determined.

One rich source of various progenitor cell types is embryo-derived stem cells, which have the capacity to differentiate into phenotypes of all somatic and germ cell lineages. Under appropriate conditions, ES cells are derived from undifferentiated cells of mouse [24] and human [25] blastocysts. Once established, ES cell lines consist of undifferentiated, pluripotent cells with a nearly unlimited self-renewal capacity in vitro (reviewed in [26]). Routinely, differentiation of ES cells is induced by the formation of embryoid bodies (EBs [27, 28]). During EB formation, an outer layer of endoderm-like cells is formed followed by the development of an ectodermal 'rim' and subsequent specification of mesodermal cells. Plating of these EBs onto adhesive substrates allows continued differentiation into a variety of specialized cell types of ectodermal, mesodermal and endodermal lineages. The temporal expression of tissue-specific genes and proteins in vitro indicates that early processes of embryonic development are recapitulated in vitro, and necessarily involve the generation of progenitor cells (reviewed in [1, 2]).

Cellular differentiation in vitro and in vivo is closely connected with morphological changes based on intermediate filament (IF) protein remodelling. Intermediate filaments are expressed in cell-type-specific patterns following and demarcating pathways of embryonic development and cellular differentiation [29]. The IF protein nestin, which was initially identified as a marker of neural stem and neural progenitor cells [30, 31], shows a wider range of expression than previously thought (table 2).

Here, we demonstrate that nestin is abundant in early ES-derived progenitor cells that proliferate and differentiate into the neuroectodermal, mesodermal and endodermal lineages, suggesting that nestin may be a common marker of multi-lineage progenitor cells. Moreover, the demonstration of nestin expression both in embryonic and in adult tissues after injury suggests that nestin-expressing cells may be directly implicated in tissue regeneration.

Gene and protein structure and expression pattern of nestin

Nestin was first identified in neuroepithelial stem cells of rat [31], but since then human [32] and mouse [33] nestin genes have been cloned. The nestin genes show structural similarities to type III (e.g. vimentin, desmin) and especially type IV (e.g. neurofilaments, α-internexin) IF proteins [29], which led to its classification as a type IV IF protein. Due to a low degree of protein sequence homology of the core domain in comparison to the five IF protein classes, in addition nestin was defined as a new type, class VI, of IF proteins [34].

As is typical for IF proteins, nestin is characterized by an α-helical central 'rod' domain that contains repeated hydrophobic heptad motifs. The 240-kDa protein nestin contains a short N-terminus and an unusually long C-terminus, which interacts with other cellular components, such as microfilaments and microtubules [31]. Nestin is unable to self-assemble [29], most likely because of its very short N-terminus (a domain necessary for IF assembly); therefore, nestin requires the presence of other IF proteins, such as vimentin [35] or desmin [36], to assemble into heterodimers and mixed polymers (see fig. 1b).

The rat 5945-bp nestin gene [31] contains five exons spanning four introns [www.ensembl.org, see fig. 1a], whereas the mouse nestin gene contains eight exons with a coding sequence of 5454 bp (www.ensembl.org). Transcriptional regulation of the nestin gene is unique. Analyses of the rat nestin promoter in transgenic mice indicate that the region upstream of the first exon does not contain any identifi-

Table 1. Proliferation capacity of ES-derived nestin-positive cells in vitro.

Cultivation stage	Nestin+/desmin–		Nestin+/desmin+	
	Total cell number (%)	BrdU labelling (%)	Total cell number (%)	BrdU labelling (%)
4 + 10 d	41.6 ± 10.7	28.4 ± 12.3	31.4 ± 8.2	45.3 ± 28.5
4 + 14 d	15.8 ± 5.4	4.2 ± 2.2	10.2 ± 3.7	6.9 ± 3.4

R1 ES cells were cultured in the expansion phase of nestin-positive cells according to [43]. The BrdU incorporation into nestin-positive (nestin+/desmin–) and nestin and desmin co-expressing (nestin+/desmin+) ES-derived cells was analysed at 10 and 14 days after plating of 4-day-old EBs (= 4 + 10 d and 4 + 14 d). BrdU incorporation was evaluated after 12 h BrdU (10 µmol l^{-1}) treatment by triple-immunofluorescence staining (anti-BrdU antibody, 1:50, abcam UK; rat 401, 1:3, Developmental Studies Hybridoma Bank USA; anti-desmin antibody, 1:100, Dako USA) and determined in relation to the whole cell population estimated in phase contrast. The percentage values of total cell numbers of nestin+/desmin- and nestin+/desmin+ cells were determined by phase contrast microscopy. Mean values ± SD are given.

Table 2. Nestin expression in embryonal and fetal tissues of mouse (m), rat (r), and human (h).

Nestin-expressing cell types	References	Methods
Neuronal precursor cells	(r) [5]	immunohistochemistry
Radial glia cells	(r) [5, 103] (h) [82]	immunohistochemistry
Schwann cells	(r) [103, 104]	immunohistochemistry
Neural crest cells	(r) [103] (m) [68]	immunohistochemistry
Oligodendrocyte precursors	(r) [105]	immunohistochemistry
Developing skeletal muscle cells	(r) [31, 69, 103] (m) [106]	immunohistochemistry Northern blotting
Developing cardiomyocytes	(m) [107]	immunohistochemistry Western blotting RT-PCR
Presomitic mesoderm	(m) [37]	nestin-lacZ transgene expression
Myotome	(r) [103] (m) [37, 106]	immunohistochemistry
Dermatome	(m) [106]	immunohistochemistry
Mesonephric mesenchyme	(r/m) [108]	immunohistochemistry
Myoid cells	(r/m) [108]	immunohistochemistry
Dental lamina, dental epithelium, ectomesenchyme in dental papilla, enamel organ, dental follicle, stratum intermedium, pulp	(m) [109]	immunohistochemistry In situ hybridization
Endothelial cells of developing blood vessels	(r) [110]	immunohistochemistry
Vascular endothelium in developing pancreas	(r) [72] (m) [74, 76]	immunohistochemistry
Pancreatic epithelial progenitor cells	(m) [75, 76]	immunohistochemistry
Epithelium of lens vesicle	(r) [111]	immunohistochemistry
Retina (Müller cells)	(h) [112]	immunohistochemistry
Hepatic oval cells	(h) [113]	immunohistochemistry

able regulatory elements [37]. In fact, nestin expression in muscle precursor and neuroepithelial stem cells of the central nervous system (CNS) is independently regulated by temporally and spatially restricted enhancer elements in the first and second introns, respectively [37]. The second intron contains two highly conserved elements, a midbrain and a CNS enhancer element that are activated and independently regulated in rat and human [38, 39] (see fig. 1a) and which are not strong enhancers in the peripheral nervous system [37]. The CNS enhancer is active in neural stem cells of the developing CNS (as shown also for the human nestin enhancer that is activated at E10.5 of mouse development [40]), whereas the midbrain enhancer is active in the developing midbrain [38, 39]. The midbrain enhancer element consists of a nuclear hormone receptor binding site and a more 3′ tissue-non-specific transcriptional regulator element that interacts with an unknown binding factor [41] (see fig. 1a). In the CNS enhancer, one nuclear hormone receptor binding site regulates expression in telencephalon and dorsal mesencephalon. From the two POU-domain binding sites that were identified [38, 39, 42], only the downstream POU site is required for full CNS-specific expression [42].

Only limited data are available on the transcriptional regulation of nestin in other tissues or in vitro. Lineage-tracing experiments have shown that transient nestin expression can be detected in pancreatic epithelial progenitor cells (see below), but to date, almost nothing is known about transcriptional regulation of nestin via lineage-restricted differentiation and development. We have therefore used the in vitro ES cell differentiation system, which in many respects mimics early stages of development to elucidate the expression profile of nestin and to predict its functional role during differentiation and development.

Properties of nestin-positive cells derived from ES cells

Proliferation of ES-derived nestin-positive cells in vitro

A characteristic property of progenitor cells is their high, but finite proliferation capacity. To analyse the proliferative capacity of nestin-expressing cells, we determined bromo-deoxyuridine (BrdU) incorporation in ES-derived

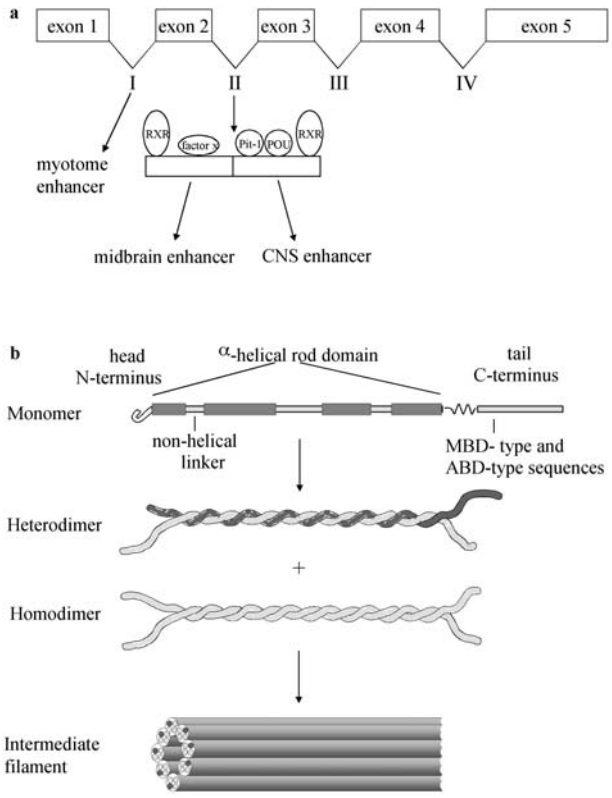

Figure 1. Gene and protein structure of nestin. (*a*) The rat nestin gene consists of five exons encompassing four introns (I, II, III, IV). Enhancer elements acting during myotome and brain development are located in the first and second introns, respectively. In the second intron three different nuclear receptor binding sites (RXR: RXR-β-half site; factor x: unknown factor) and two POU binding sites (Pit-1, POU class III factor) were identified. (*b*) Nestin, a 240-kDa protein (monomer) is characterized by a short N-terminus (11 amino acid residues) and an unusually long C-terminus (>1400 amino acid residues) [31]. Nestin (dark) forms mainly heterodimers with vimentin (light), the latter assembling into homodimers. Intermediate filament proteins assemble into heterodimers, homodimers or form mixed polymers (in the case of nestin intermediate filaments, nestin could be located at the periphery as proposed [29]). MBD, microtubule binding domain; ABD, actin binding domain.

cells cultured under conditions that support the proliferation of nestin-positive cells. Two time points representing early and late stages of the expansion of nestin-expressing cells were analysed (for methods, see [43, 44]). Under these conditions, we detected two nestin-positive phenotypes that were labelled either by nestin or by both nestin and desmin, an IF protein typical for mesodermal and myogenic cells, respectively. Therefore, BrdU labelling of nestin-expressing desmin-positive and desmin-negative cells, respectively, was separately analysed (table 1).

Maximum BrdU incorporation was detected in nestin-positive/desmin-positive cells during early expansion of nestin-expressing cells (= 4 + 10 d, 45.3%). Labelling decreased with continued cultivation until stage 4 + 14 d (6.9%). A relatively lower BrdU labelling frequency was measured in nestin-positive/desmin-negative cells at early stages (= 4 + 10 d, 28.4%), but again, the number of nestin-positive, BrdU-positive cells decreased (4.2%) with cultivation time (up to 4 + 14 d). These data demonstrate that nestin-expressing (and nestin/desmin-expressing) cells have limited proliferation potential consistent with a progenitor cell phenotype that has committed to differentiate [3] (see table 1). Similar values of ES-derived BrdU-labelled nestin-positive cells at the expansion stage have been described [45]; however, the authors did not discriminate between nestin- and desmin-expressing cell fractions.

We therefore suggest that ES-derived nestin-positive cells have (i) limited proliferation potential and (ii) potentially high plasticity (nestin and desmin expression).

Nestin expression during ES cell differentiation in vitro

To analyse how nestin expression is activated in ES cells committed to early steps of differentiation, and to determine whether nestin might be a marker of early progenitor cells, we studied nestin expression in ES-derived cells during in vitro differentiation. Nestin was abundant in embryoid bodies (EBs) and early EB outgrowths. A significant number of nestin-positive cells was detected in day 2 and day 6 EBs (fig. 2a–c) with a significant concentration of nestin-positive cells at the 'ectodermal rim' of 2 d EBs (fig. 2a). In EB outgrowths after plating, nestin-positive cells show a spindle-shaped morphology at the periphery of the colonies, suggesting nestin expression in migrating and proliferating cells (fig. 2d).

After spontaneous differentiation or specific differentiation induction into neural, pancreatic and hepatic cell lineages (for methods, see [28, 43, 46, 47]), we observed a partial and transient co-expression of nestin with lineage-specific marker proteins, including glial fibrillary acidic protein (GFAP, neuroglial cells, fig. 2e), C-peptide (pancreatic cells, fig. 2f), albumin (hepatic cells, fig. 2g) and desmin (mesenchymal/mesodermal cells, fig. 2h). The co-expression of nestin with lineage-specific marker proteins was only found at time points that define the onset of cell lineage specification before terminal differentiation. With continued differentiation into specialized cell types, nestin expression was downregulated in all differentiated cells.

Our findings suggest that the appearance and timing of nestin expression are consistent with the formation of lineage-specific progenitor cells or precursors of the neural, pancreatic and hepatic lineages.

ES-derived neural differentiation

The first evidence of neuronal differentiation of ES cells in vitro was reported during the late 1980's [48]. Spontaneous differentiation of ES cells typically generates low numbers of neuron-like cells [49, 50], but several strategies have been adapted to increase the number of neuron-

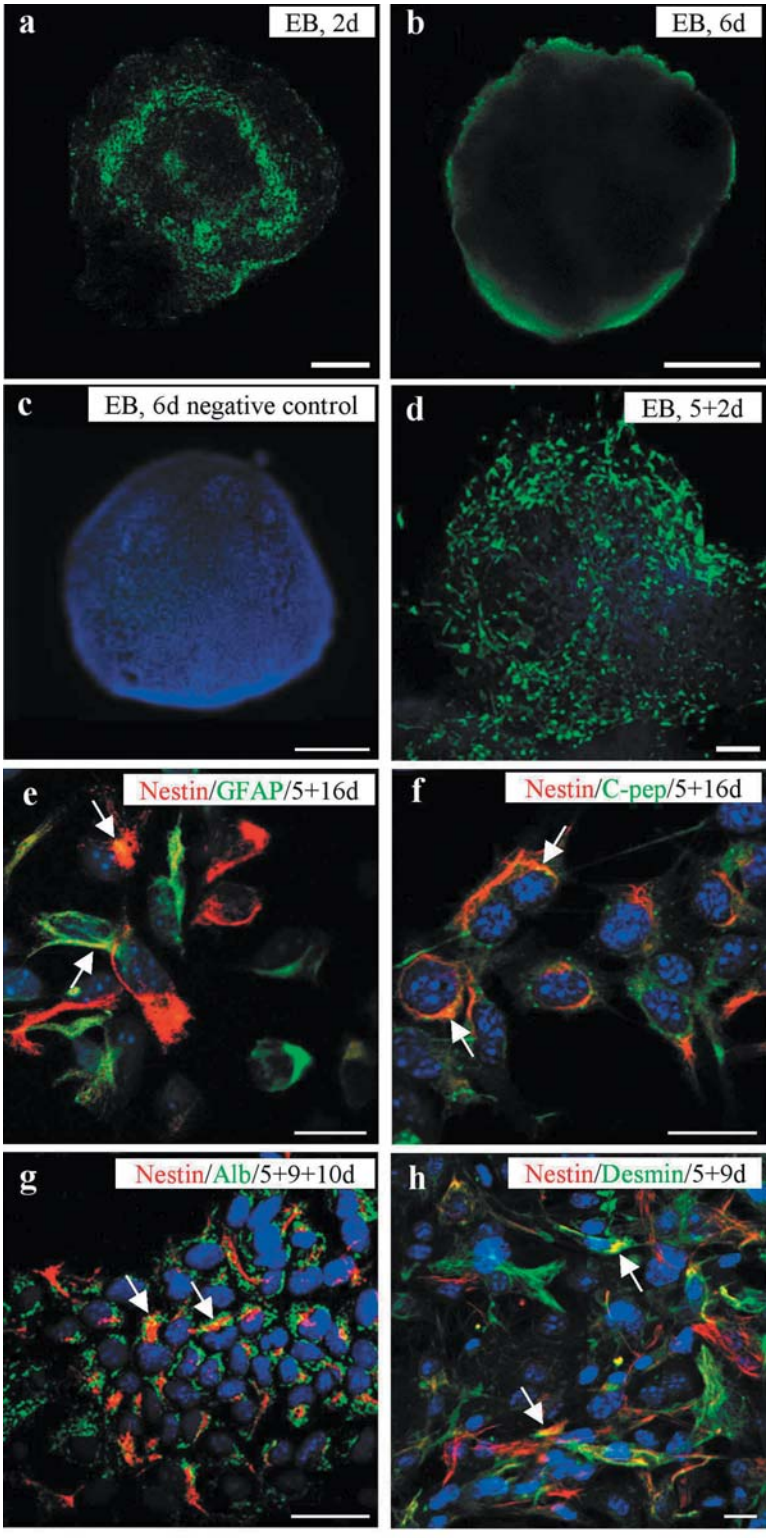

Figure 2. Abundance of nestin in different progenitor cell types during ES cell differentiation (for ES cell cultivation, see [28, 44]). (*a–d*) Indirect immunofluorescence analysis [47] of (*a*) 2-day- and (*b*) 6-day-old embryoid bodies (EBs) in suspension, of (*c*) control (primary antibody was omitted) and (*d*) a 5-day-old EB 2 days after plating. (*e–h*) Double immunofluorescence images of different progenitor cell types expressing (*e*) glial fibrillary acidic protein (GFAP, green, clone G-A-5, 1:20, Roche Molecular Biochemicals, Germany) and nestin (red, clone rat 401, 1:3, Developmental Studies Hybridoma Bank, USA), (*f*) C-peptide (C-pep, green, 1:50, Linco Research Inc., USA) and nestin (red), (*g*) albumin (Alb, green, 1:100, Serotec, USA) and nestin (red) and (*h*) desmin (green, 1:100, Dako, USA) and nestin (red). Hoechst 33342 (blue) was used to visualize cell nuclei. Bar, 20 µm (*e*, *g*, *h*), 30 µm (*f*), 50 µm (*a*), 60 µm (*d*), 270 µm (*b*, *c*). Arrows point to areas of co-expression (yellow).

like cells, including differentiation induction with retinoic acid (RA [49–51]), genetic [52] and growth factor-mediated (basic fibroblast growth factor, bFGF; epidermal growth factor, EGF; FGF8 and sonic hedgehog, Shh [45, 53, 54]) lineage selection (including reporter gene expression and fluorescent activated cell sorting (FACS) selection [55]), and by stromal cell-derived inducing activity ([56], for review, see [57]). Neuronal differentiation is specifically induced by the addition of neuronal differentiation factors including neurotrophic factors and ascorbic acid [54] and survival promoting factors, such as interleukin (IL)-1β, glial cell line-derived neurotrophic factor (GDNF), neurturin and transforming growth factor (TGF)-β3 [43]. High concentrations of RA applied at early stages of EB formation also result in the induction of neural-specific genes, proteins, ion channels and receptors in a developmentally controlled manner [49–51, 58]. Under these conditions, we find nestin-positive neuro-glial progenitor cells as early as 3 days after the onset of differentiation, indicating that ES cells at first differentiate into nestin-expressing cells and later into neuronal and glial cells [50].

ES-derived pancreatic differentiation

Spontaneous differentiation of ES cells in vitro also results in endodermal cell types that include a low number of cells (0.1%) which display characteristics of pancreatic endocrine insulin-producing cells [59]. This proportion has been markedly increased by the selection of nestin-positive progenitor cells [60] through a method that involves the formation of EBs, selection and propagation of progenitors expressing nestin and finally cell maturation that results in the formation of insulin-positive cell clusters. The clusters release insulin in vitro, but fail to normalize high blood glucose levels after transplantation into diabetic mice [60]. A reinvestigation of this protocol indicates that the differentiated cells were insulin-positive, but insulin messenger RNA (mRNA) levels were weak, and C-peptide, a by-product of de novo insulin synthesis, and secretory granules were not detected [61]. The insulin-positive cells were characterized by small condensed nuclei and signs of apoptosis. These cells had evidently taken up the hormone from the culture medium, rather than producing insulin themselves [61].

We have, however, modified this protocol [60] in two fundamentally important ways. First, we expanded nestin-positive cells in the absence of bFGF (as used for expansion of neural progenitor cells) and second, we treated cells with pancreatic differentiation factors (such as nicotinamide, insulin and laminin) for 19 instead of 6 days. Under these conditions, ES cells readily generate insulin-producing cells via nestin-positive progenitors [46]. By applying the modified protocol with ES cells that constitutively express Pax4 (Pax4+), a four- to five-fold increase in the number of insulin-expressing cells that contain secretory granules, which were similar to embryonic beta cells, were found. Transplantation of these Pax4+ ES-derived cells rescued diabetes in streptozotocin-treated diabetic mice ([46], P. Blyszczuk et al., unpublished data). The successful differentiation of ES cells into insulin-producing cells via nestin-positive progenitor cells has also been described following treatment with a phosphoinositide 3-kinase inhibitor [62].

Although, the differentiation method based on the selection of nestin-positive cells [60] does not efficiently generate functional pancreatic cells from ES cells, our results show that the differentiation of ES cells into functional insulin-producing pancreatic cells occurs via a transient stage of progenitor cells expressing C-peptide and nestin (fig. 2f).

ES-derived hepatic differentiation

ES cells readily differentiate into functional hepatocyte-like cells in vitro [63–66]. Recently, we found that ES cells, when cultured via EBs in growth factor-supplemented, but serum-free medium, differentiate into hepatocyte-like cells via intermediates that express nestin. These nestin-positive cells could then be directed into hepatic cell types by a cocktail of differentiation factors [47].

Also, spontaneous differentiation of ES cells without selection for nestin-expressing cells results in hepatic cells that develop from nestin-positive progenitor cells co-expressing albumin (fig. 2g). These cells differentiate into hepatocyte-like cells that express liver-specific genes and proteins and acquire a functional hepatic phenotype as demonstrated by albumin secretion (G. Kania et al., unpublished data, [44]).

Altogether, the data from ES cell in vitro differentiation models suggest that at least neural, pancreatic and hepatic cells are all derived via nestin-positive intermediates. Because this represents an in vitro system, the question remains whether nestin is expressed in progenitor cells associated with embryonic development or tissue regeneration.

Nestin expression during embryonic development

Nestin is widely expressed during mammalian embryogenesis in a variety of embryonal and fetal tissues (see table 2). It is first detected in the one-cell layer of neural ectoderm at day E7 [67], in neuroepithelial cells of E7.75 embryos [68], in presomitic mesoderm and in the myotome layer of the somites in mice [69]. Importantly, nestin is abundant during early neurogenesis [5] and in the peripheral and central nervous systems, suggesting that nestin-positive cells are critical for embryonic neural development. As shown in table 2, nestin expression is most prominent in tissues of the neural system, but it is also present in other embryonal and fetal cell types in mouse, rat and human.

Although nestin expression is unequivocally accepted as a marker of neural progenitor cells, the expression of nestin during pancreatic development remains controversial. Nestin is present in a subset of (insulin-negative) cells in pancreatic islets, suggesting that it might be a marker of pancreatic precursor cells [70]; however, Selander and Edlund [71] did not find nestin in the pancreatic ductal epithelium, where the potential progenitor cells reside. Instead, nestin was restricted to mesenchymal cells, the so-called pancreatic stellate cells [71, 72]. The observation that nestin is expressed in pancreatic vascular endothelial cells, which are closely associated with endocrine cells [73, 74], also suggests that nestin-lineage cells may play a role in the growth and maintenance of islets.

Recently, lineage-tracing studies were performed using nestin regulatory elements to drive Cre-mediated activation of the LacZ reporter gene in the developing pancreatic epithelium [75, 76]. Nestin was transiently expressed in pancreatic epithelial progenitor cells in E10.5 mouse pancreas [75], but later, nestin-expressing cells were found to contribute mainly to the exocrine lineage [75, 76]. In adults, nestin was detected only at low levels in endocrine cells [77]. These experiments demonstrate that (i) a nestin promoter/enhancer element containing the second intron of the rat nestin locus is temporarily active in E10.5 pancreatic epithelial (progenitor) cells, (ii) these nestin-positive precursors contribute mainly to the formation of exocrine acinar cells, and (iii) nestin-positive epithelial progenitors are involved during pancreatic development and may be regulated by specific EGF receptor activity [75, 76]. At this time, it remains unclear whether nestin-positive progenitor cells are involved in hepatic development.

Nestin expression in adult tissues

In adult organisms, nestin-expressing cells are restricted to defined locations (table 3), where they may function as a (quiescent) cellular 'reserve' capable of proliferation, differentiation and migration after re-activation. Nestin is also expressed in several mature cell types such as adrenocortical cells and interstitial cells of Cajal (see table 3); however, it remains unclear what role, if any, nestin plays in cells that do not migrate or proliferate in the healthy organ.

After injury, it has been observed that cells re-express or upregulate nestin (e.g. [78, 79]), which may indicate extensive remodelling or a reversion to a more immature phenotype. As an example of the latter, nestin expression is reactivated in astrocytes [78, 80], indicating that signalling processes of early neuronal development are recapitulated. Indeed, nestin is not only upregulated in response to injury in the CNS, but also in liver, pancreas, skeletal muscle, as well as in the CNS, gastrointestinal tract and odontoblasts under pathological conditions (see table 3).

During tumourigenesis, cells of certain tissues show protein patterns characteristic for stem/precursor cells of the tissue. In CNS tumours, in particular in malignant tumours, nestin expression was found [81, 82]. This property, which is correlated to high proliferation and migration activity of malignant cells, suggests that nestin might be useful in tumour diagnosis.

The molecular mechanisms of nestin re-activation after injury are not well understood. Independent mechanisms, including extracellular factors, cell-cell interactions, transcriptional regulation and intermediate filament remodelling, may be involved:

(i) Extra-cellular soluble factors may trigger gene activation after their release from adjacent tissues [83]. Examples for such factors are nerve growth factor or acidic fibroblast growth factor involved in the regeneration of neural cells in the brain [84] or the involvement of bone morphogenetic protein-4 (BMP4) in the regeneration of dental pulp cells [85].

(ii) Up-regulation of nestin could be induced by changes in cell-cell contact patterns. As shown for primary astrocytes, loss of intercellular contacts resulted in strong nestin expression [86].

(iii) Transcriptional regulation may be involved in nestin expression, similar to that described for adult CNS stem cells under normal conditions and after injury. Johansson and co-workers have additionally found that elements located outside the most conserved region of the second nestin intron are required for activation after brain and spinal cord injury [87]. These findings suggest that complex gene regulatory events may be activated after cellular damage and regeneration.

(iv) Similar to that seen in early development, IF protein remodelling may be involved in regenerating cells after injury. In neuroepithelial cells of the neural tube, for example, nestin plays a role in the organization and maintenance of the elongated cell morphologies [88]. Nestin also mediates phosphorylation-dependent disassembly of vimentin IF during mitosis in a concentration-dependent manner [89].

All these results support our view that nestin expression may be involved in tissue regeneration in adults.

Nestin expression in somatic cells cultured in vitro

In addition to nestin expression during embryogenesis and in adult tissues, numerous cell types express nestin after in vitro cultivation. This could suggest that nestin expression may be a consequence of in vitro cultivation conditions that are not necessarily representative of in vivo expression dynamics. Cell types which demonstrate this expression phenomenon in vitro include bone mar-

Table 3. Nestin expression in adult and injured tissues of mouse (m), rat (r) and human (h).

Nestin-expressing cell type	References	Methods
Normal adult tissues		
Satellite cells in dorsal root ganglia	(r) [103]	immunohistochemistry
Schwann cells	(r) [103, 104] (r/m) [86]	Immunohistochemistry
Brain:		
Subependymal cells (forebrain)	(m) [114] (r) [83, 115]	immunohistochemistry
Ependymal zone	(m) [116, 117]	immunocytochemistry
Subventricular zone	(m) [116, 117]	immunocytochemistry
Dentate gyrus	(m) [118] (h) [119]	immunohistochemistry
Olfactory neuroepithelium/olfactory bulb core	(r) [120] (r/m) [121]	immunohistochemistry Western blotting
Pericytes and periendothelial cells of parenchyma	(m) [122]	immunohistochemistry
Substantia nigra	(m) [123]	immunohistochemistry
Retina (peripapillary, posterior, equatorial, peripheral anterior retina and epiretinal membran)	(h) [124]	immunohistochemistry
Dermis-derived progenitors (SKPs)	(m) [98]	immunohistochemistry
Pancreatic stellate cells	(r) [72]	immunohistochemistry
Pancreatic endothelial cells	(r) [72] (h) [73] (m) [74]	immunohistochemistry immunohistochemistry
Cerebral and spinal (cord) endothelial cells	(h) [81] (r/m) [86] (r) [110]	immunohistochemistry
Interstitial cells of Cajal (gastrointestinal tract)	(h) [125, 126]	immunohistochemistry
Muscularis propria (gastrointestinal tract)	(h) [126]	immunohistochemistry
Hair follicle outer-root sheath progenitor cells	(m) [127]	immunohistochemistry
Adrenocortical cells and adrenomedullary chromaffin cells	(r) [128]	immunohistochemistry Western blotting
Cardiac progenitor cells	(h) [129]	immunohistochemistry
Optic nerve	(m) [130] (h) [124]	immunohistochemistry immunohistochemistry
Sertoli cells	(r/m) [108]	immunohistochemistry
Odontoblasts	(m) [109]	immunogold labelling
Injured adult tissues		
Reactive astrocytes following unilateral intraventricular kainic acid lesion	(r) [78, 83, 131]	immunohistochemistry
Reactive astrocytes following focal cerebral ischemia	(r/m) [86] (r) [80]	immunohistochemistry immunohistochemistry In situ hybridization
Glial cells after spinal cord injury	(r) [132]	immunohistochemistry
Reactive astrocytes following neural grafting or brain wounds	(r) [133]	immunohistochemistry In situ hybridization
Non-reactive astrocytes in ipsilateral cortex after lesion	(r) [83]	immunohistochemistry
Ependymal cells after spinal cord injury	(r) [79]	immunohistochemistry
Endothelial cells in cerebral arteriovenous malformation	(h) [134]	immunohistochemistry RT-PCR
Pancreatic endothelial cells	(r) [72]	immunohistochemistry
Hepatic stellate cells following induction of fibrosis	(r) [135]	immunohistochemistry
Odontoblast under pathological conditions	(h) [85]	immunohistochemistry
Myoblasts after injury	(r) [136]	immunohistochemistry
Purkinje cells in Creutzfeldt-Jakob disease	(h) [137]	immunohistochemistry
Malignant tumours of CNS	(h) [81, 82]	immunohistochemistry Western blotting
Gastrointestinal stromal tumours	(h) [125]	Immunohistochemistry Western blotting Northern blotting RT-PCR

row [90, 91]-, fetal liver [92]-, cord blood [93]- and peripheral blood [94]- derived haematopoietic cells, and cultures derived from adult corneal limbal epithelium [95, 96], utricular macula cells [97], skin [98], adipose-derived stromal cells [99] and intestinal epithelium [C. Wiese et al., unpublished], several of which we now believe to have characteristics of progenitor cells.

Human islet-derived cells cultured in vitro also express nestin, proliferate and differentiate into insulin-secreting cells, and in parallel, these cultured cells express markers of ductal epithelium, exocrine pancreatic cells and hepatocytes [100–102].

Under appropriate differentiation conditions, nestin-expressing cells derived from non-neural sources such as corneal limbal epithelium [95], utricular macula cells [97] and adipose-derived stromal cells [99] could also be directed into neuronal and glial as well as non-neural phenotypes, including smooth muscle cells and adipocytes [98].

Nestin expression is therefore widespread both in vivo and after in vitro cultivation of multiple cell lineages.

Summary and conclusions

The intermediate filament protein nestin, a well-known marker of neural progenitor cells has been detected in a multitude of cellular phenotypes in embryonic and adult tissues, in vivo and in vitro. Cells expressing nestin show characteristic features of progenitor cells, such as multipotency, high proliferation, limited self-renewal and regeneration capacity. After differentiation induction of ES cells by tissue-specific growth and extracellular matrix factors, nestin expression is significantly upregulated. In a transient ES cell differentiation stage, nestin expression is found in different subpopulations of potent progenitor cells differentiating into mesenchymal/mesodermal, neural, pancreatic endocrine and hepatic cell lineages.

Proliferation, migration and a broad differentiation potential are hallmarks of nestin-expressing cells found during embryogenesis, as well as during ES cell differentiation. In adult tissues, nestin expression is most closely associated with a stem/progenitor cell population, and proliferation and migration of these nestin-positive cells appear to be reactivated in response to injury during tissue regeneration.

Although, many questions concerning the functional role and transcriptional regulation of nestin expression in various cells and tissues remain open, our ES cell data together with published findings lead us to propose nestin as a marker of multi-lineage progenitor cells. Moreover, the involvement of nestin expression not only in development, but also in tissue regeneration, could open new perspectives for the identification of cells suitable for regenerative cell therapies.

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