Lamin A: a putative colonic epithelial stem cell biomarker which identifies colorectal tumours with a more aggressive phenotype

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Abstract
Abnormalities in the expression, distribution and structural organization of A-type lamins are most commonly associated with a spectrum of inherited disorders which predominantly affect mesenchymal lineages, collectively known as laminopathies. However, a new role for lamin A has been discovered in the progression of a common epithelial cancer. CRC (colorectal cancer) patients expressing lamin A/C in their tumour tissue were found to have a 2-fold greater risk of CRC-related mortality compared with patients with lamin A/C-negative tumours. Consequently, lamin A/C is a prognostic biomarker in CRC. In vitro studies suggest that lamin A is an upstream regulator of a pathway linking actin dynamics to loss of cell adhesion, leading to enhanced cell motility and consequently increased invasive potential within a tumour. The finding that lamin A is a putative colonic epithelial stem cell biomarker suggests that the poor outcome associated with lamin A/C-positive tumours may be reflective of a more stem-cell-like phenotype. The present review discusses the link between lamin A expression and tumour progression in one of the commonest causes of cancer-related death in the Western world.

Lamins and mesenchymal stem cells
In all metazoans, a supporting proteinaceous network, termed the nuclear lamina, lies subjacent to the inner nuclear membrane. It is composed of filamentous units known as lamins, which are revealing themselves to be truly multifunctional. Beyond their architectural contribution, they have been ascribed roles in the regulation of DNA replication, cell-cycle progression, differentiation and apoptosis. Most interestingly, they appear to organize interphase chromatin and play a role in the regulation of gene expression [1,2].

Mammalian somatic cells contain both A-type (lamins A, C and A10, encoded by LMNA) and B-type (lamins B1 and B2, encoded by LMNB1 and LMNB2 respectively) lamins [3]. Although expression of B-type lamins is essential for cell viability [4], expression of A-type lamins is developmentally regulated and is understood to accompany tissue and cellular differentiation [5–7]. However, the reported involvement of A-type lamins in premature aging syndromes, such as HGPS (Hutchinson–Gilford progeria syndrome) has ignited speculation that nuclear lamins may also be important for adult stem cell function [8]. Alongside HGPS, mutations in LMNA have been assigned to an ever-increasing number of seemingly heterogeneous and rare human genetic disorders, collectively termed laminopathies [9]. Interestingly, these diseases share one common attribute in that they predominantly affect tissues of mesenchymal origin, including muscle, adipose tissue and bone. It has been reported recently that a truncated form of lamin A responsible for HGPS (progerin) overactivates the Notch signalling pathway in MSCs (mesenchymal stem cells) [10]. This led to equivalent dysregulation of cellular differentiation to that observed when the Notch pathway was constitutively activated, directly linking lamin A to essential regulators of stem cell homeostasis [10]. A significant proportion of laminopathies are muscle-wasting disorders, incorporating muscular dystrophies and cardiomyopathies, which have a severe impact on expected lifespan [11]. Investigations into the accompanying dystrophic pathologies have implicated loss of functional lamin A in a failure of muscle stem cells to self-renew or differentiate, compromising their capacity for tissue regeneration [12–14].

Lamin A and epithelial stem cells
Although research into the molecular and cellular basis of laminopathies has yielded tantalizing evidence that lamins, particularly A-type lamins, are important for MSC homeostasis [15], little is known about the relationship between lamins and adult epithelial stem cells. The colon is a good starting point for this type of investigation as it comprises an easily accessible and histologically distinct epithelium which encloses the colonic lumen. The epithelium is invaginated into thousands of flask-shaped units, termed crypts. These structures are highly dynamic and completely renew themselves every 5–7 days [16,17]. The continuous demand for differentiated cells at the top of the crypts is met by a group
of five to ten stem cells which are putatively located at the bottom of each crypt [18]. A large number of colonic stem cell biomarkers have been proposed, but whether they demarcate definitively the stem cell niche is less certain [19].

Using a panel of antibodies against A-type lamins [20,21], we have investigated the expression of lamin A/C in healthy colonic crypts by immunohistochemistry [22]. As anticipated [7,23,24], strong lamin A/C expression was detected in the differentiated region of the crypts, compared with the proliferative region lower down which did not express A-type lamins. Furthermore, lamin A/C-positive staining coincided with the putative location of the stem cell niche, and the number of cells distinguished corresponded to the predicted number of stem cells in the colonic crypt. Staining of serial crypt sections for lamin A/C and PCNA (proliferating-cell nuclear antigen), an S-phase marker, revealed reciprocal expression of lamin A/C and PCNA at the crypt base. PCNA was not expressed in the putative stem cell niche, whereas the proliferating/transit-amplifying region strongly expressed PCNA [22].

Analysis of the underlying genetics of laminopathies has shown that lamin A is sometimes affected independently of lamin C [9]. Using antibodies specific either to lamin A [20] or lamin C [21], differences in their expression patterns along the crypt axis have been uncovered [22]. Although both proteins were present in the differentiated compartment of the crypt, and both were absent from the proliferative zone, the lamin C-specific antibody did not stain any cells in the putative stem cell niche, whereas these cells were clearly positive for lamin A. Taken together, these findings strongly suggest lamin A is a prime candidate biomarker of colonic epithelial stem cells and raises the, as yet unanswered, question of the function of lamin A in this environment.

**CRC (colorectal cancer): a stem cell disease?**

Colorectal cancer represents one of the most prevalent cancers in the Western world, accounting for 412,900 incident cases and 207,400 deaths in Europe in 2006, placing it just behind lung cancer as one of the commonest causes of cancer-related death [25]. CRC is known to develop over many years through a series of clinicopathologically distinct stages; however, current anatomic staging criteria are simply unable to provide detailed predictions about individual patient outcome. In short, a system which could more accurately predict the likelihood of tumour progression and therefore prognosis would be a hugely beneficial development.

When trying to tease out the mechanisms behind colorectal tumour development and advancement, it is important to consider the potential involvement of stem cells. Increasingly, researchers are considering CRC to be a disease of stem cell dysfunction. The 'bottom-up' model of CRC development explains this idea [26]. This model states that CRC develops from a single genetic hit to a stem cell. In sporadic CRC, the first hit is widely thought to be to APC (adenomatous polyposis coli) which is a crucially important component of the Wnt signalling pathway [27,28]. Absence of APC constitutively activates β-catenin, allowing it to translocate to the nucleus, thereby switching on proliferation. The resulting dysplastic cells migrate upwards, eventually forming a stalk-like protrusion into the lumen, known as a polyp. Accumulation of mutations results in an increase in tumour size and, eventually, the development of a full-blown carcinoma [29].

The 'top-down' model takes a slightly different viewpoint [26]. It states that CRC develops from a mutation at the top of the crypt. The dysplastic cells then migrate downwards. Acquisition of further mutations causes the tumour to become more pathogenic until the requisite number of mutations are achieved, giving rise to a full-blown carcinoma. The 'top-down' model suggests either that the stem cell niche resides at the top of the crypt which is opposite to the general consensus, or that CRC is the result of a mutation in a differentiated cell which is then set on a path of dedifferentiation whereupon it becomes progressively more stem-cell-like.

**Lamin A and colorectal tumour progression**

We have recently undertaken a significantly large retrospective study to examine immunohistochemically the expression pattern of lamin A/C, now a putative stem cell biomarker, in CRC [22]. Tumour material (n = 656) was randomly selected from an archive of CRC cases involved in the Netherlands cohort study on diet and cancer [30] and double-blind scored for lamin A/C expression. Samples were associated with 10-year follow-up data showing there were 163 CRC-related deaths within that time. Noticeable differences in lamin A/C expression in tumour tissue were analysed alongside accompanying clinicopathological, biological and epidemiological data, revealing a counter-dogma effect of A-type lamin expression in CRC. Cox hazard ratio analysis indicated that patients with lamin A/C-positive tumours were almost twice as likely to suffer CRC-related death compared with patients lacking the biomarker. The protective effect of lamin A/C-negative status was independent of any other confounding factors such as patient age at diagnosis, family history, tumour location, APC or TP53 (tumour protein 53) status. This study unexpectedly associated lamin A/C expression with a more aggressive tumour phenotype and suggested that lamin A/C is an independent predictor of tumour recurrence and likelihood of CRC-related mortality.

Downstream investigations showed that ectopic expression of lamin A in a Dukes’ B CRC cell line known to lack the endogenous protein increased transcription of the actin-bundling protein T-plastin, which in turn resulted in the down-regulation of E-cadherin [22]. This revealed a potential mechanism by which lamin A may influence tumour progression. Plastins are known modulators of the actin cytoskeleton and have been implicated in invasion and metastasis [31,32]. E-cadherin is an essential cell adhesion molecule in healthy tissues, and its loss has been correlated with invasive behaviour in neoplastic tissues and carcinoma development [33]. The observation that expression of lamin A increased cell motility, whereas siRNA (small interfering RNA)
knockdown of T-plastin in lamin A/positive cells resulted in a significant reduction in motility, suggested to us that lamin A is an upstream regulator of a pathway that links changes in actin dynamics and loss of cell adhesion to increased cell motility and, consequently, a more invasive tumour phenotype [22].

The relationship between lamin A expression in colonic stem cells and colorectal tumour development: a perspective

The unanticipated connection between lamin A/C expression and potentially more aggressive colorectal tumours seems, at first look, counter-intuitive. Differential expression of nuclear lamins, particularly lamins A and C, has been reported in epithelial, lymphoid and mesenchymal tumours [23,34–37]. However, the down-regulation of lamin A/C was generally associated with neoplasia. Absence of A-type lamins in tumours had been correlated with both a more proliferative phenotype and poor differentiation status [21,38]. Furthermore, abrogation of lamin A/C expression as a result of hypermethylation of LMNA had been associated with poor survival in patients with nodal diffuse large B-cell lymphomas, albeit in a limited number of samples [39]. However, it is the intriguing finding that lamin A identifies specifically the putative epithelial stem cell niche of the colon which alludes to the possible reasons for our unexpected findings in CRC.

Central to the ‘bottom-up’ model of colorectal tumour histogenesis is the idea that colorectal adenomas are initiated in the stem cells, after which subsequent genetic mutations are required for tumour progression [26]. Furthermore, the attractive cancer stem cell model of tumour development suggests that a discrete population of ‘tumour-initiating cells’ drives neoplastic development. These cells are likened to stem cells because they possess the ability to self-renew and differentiate, thereby creating a homogeneous tumour. Yet, by virtue of self-maintenance, their numbers and, accordingly, their aggressive properties are retained within a tumour, facilitating tumour expansion and spread [40,41]. In short, this theory suggests that cells with a stem-cell-like phenotype are likely to be more aggressive. The discovery that lamin A promotes a more invasive potential attests to the idea that more aggressive CRC tumours possess more stem-cell-like properties [22]. This work inevitably raises many intriguing questions, including: how is the function of lamin A modulated so that it can be expressed in apparently healthy colonic tissues and particularly the stem cells, yet correlate with poor prognosis when detected in tumour cells? It would also be interesting to investigate whether A-type lamins distinguish other epithelial stem cell niches and whether expression of lamin A/C in other epithelial tumours correlates similarly with poor outcome.

References

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