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Review

The mechanical microenvironment in cancer: How physics affects tumours

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ABSTRACT

The tumour microenvironment contributes greatly to the response of tumour cells. It consists of chemical gradients, for example of oxygen and nutrients. However, a physical environment is also present. Apart from chemical input, cells also receive physical signals. Tumours display unique mechanical properties: they are a lot stiffer than normal tissue. This may be either a cause or a consequence of cancer, but literature suggests it has a major impact on tumour cells as will be described in this review. The mechanical microenvironment may cause malignant transformation, possibly through activation of oncogenic pathways and inhibition of tumour suppressor genes. In addition, the mechanical microenvironment may promote tumour progression by influencing processes such as epithelial-to-mesenchymal transition, enhancing cell survival through autophagy, but also affects sensitivity of tumour cells to therapeutics. Furthermore, multiple intracellular signalling pathways prove sensitive to the mechanical properties of the microenvironment.

It appears the increased stiffness is unlikely to be caused by increased stiffness of the tumour cells themselves. However, there are indications that tumours display a higher cell density, making them more rigid. In addition, increased matrix deposition in the tumour, as well as increased interstitial fluid pressure may account for the increased stiffness of tumours.

Overall, tumour mechanics are significantly different from normal tissue. Therefore, this feature should be further explored for use in cancer prevention, detection and treatment.

1. Introduction

Tumour cells exhibit a number of specific characteristics that distinguish them from normal, healthy cells. In their seminal paper from 2000, Hanahan and Weinberg formulated six of these hallmarks [1]. One of the major features of cancer is the uncontrollable and infinite proliferation of cells. This means that cancer cells have obtained self-sufficiency in growth signals, are insensitive to growth-inhibitory signals and continuously escape death (apoptosis). Supporting this constant growth requires increased supply of oxygen and nutrients. Therefore, cancer cells require continuous formation of new blood vessels (angiogenesis). Another characteristic of cancer is the ability of tumour cells to spread through their host, invading surrounding tissue and forming metastases at distant sites. As we make progress in cancer research, new hallmarks are being elucidated and defined. In 2011, a further four hallmarks were described by Hanahan and Weinberg in their 'next generation' paper [2]. When becoming cancerous, cells acquire features that are primarily focussed on cell survival. Cancer cells are capable of reprogramming their energy metabolism allowing them to survive the often-harsh conditions of the tumour microenvironment. In addition the host's immune system, which may destruct cancer cells is evaded, for example by alterations in the glycocalyx [3,4]. Tumours also promote inflammation in the host, which is thought to support tumour growth [5]. All these alterations inside cancer cells go hand in hand with a frequently unstable genome (genomic instability), characterized by increased mutation rates chromosomal rearrangements and aberrant chromosome numbers.

As we learn more about the characteristics of cancer cells, more hallmarks will undoubtedly be defined. Increasing evidence suggests that one such hallmark is the rigidity of the tumour microenvironment [6]. Already since ancient Egyptian times, it has been observed that tumours are a lot stiffer than the surrounding healthy tissue [7,8]. This increased rigidity is the reason why palpation remains an important tool for cancer detection. Furthermore, radiological examination, such as mammography, reveals

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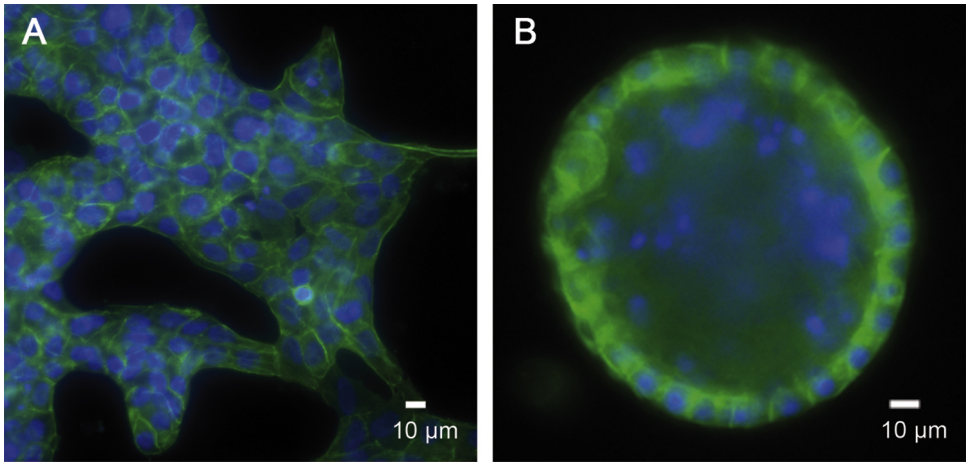

Fig. 1. Morphology of normal mammary epithelial cells is controlled by the underlying substrate. On tissue culture plastic (A) MCF10A cells form a monolayer, whereas on a thin layer of basement membrane matrix, (B) MCF10A cells form growth arrested hollow acini within two weeks. Nuclei are stained in blue, filamentous actin in green.

that cancer lesions are denser than surrounding tissue. This is a consequence of increased deposition of extracellular matrix proteins and was also shown to be an independent prognostic factor for breast cancer as well as non-small cell lung cancer [9,10]. These data suggest that the denseness of the microenvironment steers tumourigenesis.

The last decade has seen a major realization that the mechanical microenvironment with its physical stimuli affects cells as profoundly as chemical signals do. Especially matrix rigidity is highly influential on cell behaviour. In cancer, this means the birth of an entirely novel approach: physical oncology. It appears that solid tumours have extraordinary physical properties, which may prove vital for cancer detection as well as therapeutic decisions. The existence of a mechanical microenvironment to which cells, both normal and transformed, are exposed may lead to altered intracellular signalling events, contributing to carcinogenesis, cancer progression and determining the tumour response to therapy. In this review we will illustrate how vast the influence of the physical properties of the matrix in cancer is.

2. The stiffness of the matrix profoundly influences overall cell morphology and behaviour

Multiple parameters can be used to describe and quantify the mechanical properties of the materials, tissues and organs present in our bodies [11,12]. In terms of stiffness, the tissues inside our bodies cover a wide range. Brain tissue for example is very soft (<1 kPa), whereas at the other end of the spectrum bone is the hardest tissue in our body (~100 kPa) [12,13]. Nevertheless, in vitro cell culture is generally performed on glass or polystyrene surfaces, which have rigidities approximately a million times higher than the tissues from which the cells were originally isolated. These extremely stiff substrates do not sustain normal tissue morphogenesis and therefore profoundly influence cell behaviour. For example, when cultured on polystyrene surfaces normal mammary epithelial cells grow in a regular monolayer. However, when these cells are cultured on softer substrates such as basement membrane gels, they organize into characteristic well-differentiated, acinar structures, which are growth arrested, polarized, have a hollow lumen inside and form a basement membrane [14]. This is shown in Fig. 1.

Other cell types also proved highly responsive to their mechanical microenvironment. For example, fibroblasts cultured on collagen-coated polyacrylamide gels of different stiffness were able to stretch significantly more on the stiff substrates (~70 kPa) than on the soft substrates (~5 kPa), but appeared less motile [15]. A subsequent study demonstrated that fibroblasts actually have a preference for stiff substrates (30 kPa vs. 14 kPa for soft substrates), a phenomenon termed durotaxis [16]. In addition to the differences in single cell motility, substrate rigidity also affects cell–cell contact and the interaction between cells. On stiff substrates (~12 kPa), fibroblasts migrate away from each other and adopt a stretched morphology [17]. In contrast, on soft substrates (~4 kPa) cells cluster together and form tissue-like structures, in which cells have a more rounded appearance.

Apart from morphology and cell migration, substrate rigidity also profoundly influences the regulation of cell growth and apoptosis. Fibroblasts cultured on compliant substrates (~5 kPa) display reduced DNA synthesis and increased apoptosis compared to cells cultured on stiff substrates (14 kPa) [18]. Intriguingly, the response of fibroblasts after malignant transformation through H-*ras* is markedly different. These cells showed similar levels of proliferation and apoptosis on either soft or stiff substrates. This indicates that after transformation, fibroblast have overcome any difficulty growing on substrates of different stiffness. Apparently, their internal signalling pathways are extensively altered.

3. The mechanical properties of the microenvironment influences carcinogenesis, cancer progression and therapy response

3.1. Mechanical properties enable acquisition of malignancy

The mechanical properties of the tumour microenvironment are important determinants for cell behaviour. Mechanics can affect intracellular signalling events, influencing carcinogenesis, cancer progression and the tumour response to therapy. One of the prime examples illustrating the effect of the mechanical microenvironment on tumourigenesis is the behaviour of normal mammary epithelial cells in different environments. To study the response of these cells, Provenzano et al. used collagen matrices in which stiffness is regulated by matrix density [19]. In denser, stiffer matrices (~44 kPa), murine mammary epithelial cells displayed more invasive phenotypes, compared to the softer matrices of lower density (~25 kPa). Paszek et al. showed that when normal mammary epithelial cells are cultured in soft matrices of collagen and basement membrane (170 Pa), the cells organize into the characteristic organotypic mammary acinar structures [20]. However, when these cells are placed on gels with increasing stiffness (up to 1200 Pa), the normal epithelial morphology is disrupted and cells

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