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Mechanobiology of Skin

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Abstract

Although there is a general appreciation of the mechanical abilities of cells in creating the forms in nature, we understand relatively little about how the mechanobiology of cells can affect behavior. Recent studies of the effects of mechanical activity indicate that exercise and other physical perturbations can inhibit cancer progression and performance loss in aging. Thus, the tumor cell and senescent cell states are mechanically different from normal cells. New tools to measure cellular forces and the downstream biochemical changes that result from mechanical signaling have enabled the description of the matrix rigidity sensor that is missing in the vast majority of tumor cells. Tumor cells no longer form tumors upon its restoration; whereas normal cells have unregulated growth when the sensor is depleted. Further, mechanical strain of tumor cells will cause apoptosis and may have effects on keratinocyte and melanocyte tumors. This could explain some of the anti-tumor benefits of physical activity. In the case of aging, the negative effects of senescent cells on their neighbors appear to be reversed by small but not large mechanical strains in skin. Thus, cells in the tumor or senescent states respond specifically to mechanical perturbations and a deeper understanding of the important aspects of mechanobiology can be used in therapies to augment biochemical therapies to benefit the patient.

Introduction

It has been over a century since On Growth and Form (D'Arcy Wentworth Thompson, 1917) illustrated the elegant mechanics of organisms, which underlies the biodiversity in the wild. Since that time, the developments in biochemistry and molecular biology have exceeded the advancements in physical biology (physiology) at the cellular level. Only recently, the field of Mechanobiology has emerged because developments in the area of nanofabrication now make it possible to probe subcellular mechanical functions that are responsible for mechanosensing and force generation by cells to shape the tissue and ultimately the organism. What is emerging is an increasing body of evidence for multiple mechanosensing and mechanical machines within cells that are designed to create and maintain the needed mechanical properties of cells for the correct function of the tissue. When cells become cancerous or senescent, the overall system changes and the cells have different mechanical responses. Our recent studies reinforce the concept that mechanical perturbations of both cancerous and senescent cells can be beneficial.

In the case of skin, the physical parameters that are created by skin cells have been studied extensively and several of these parameters are altered in genetic diseases. What is confusing about the physical properties of skin is that skin is dynamic and yet the physical

properties are generally homeostatic. As an example, the tension in skin is normally kept constant; and when a person gains weight, the skin expands to accommodate whereas transient tugs on the skin do not cause appreciable growth. To understand the timescale of the expansion, it is useful to think about the process of getting extra skin for cosmetic surgery. One possible method is to insert a balloon in the back portion of the upper arm that is inflated over several months to produce extra skin. It is the constant elevation in skin tension over weeks to months that results in growth of new skin. Remember that the biochemical reactions responsible for controlling growth have rate constants of many reactions per second and yet the decisions to grow or not occur on an hour-day time scale. Thus, it seems that the important decisions that affect the physical parameters of the skin are made after integration of the biochemical changes from multiple mechanical inputs over time.

The Multiple Cell States in Skin Have Different Mechanical Dependencies

It is important to remember that there are multiple cell types in skin (fibroblasts, keratinocytes, etc.) that have different mechanical properties, mechanical connections and are designed to respond in different ways to common mechanical perturbations. Further, cells of each type can be in a number of cell states. We are familiar with the phases of the cell cycle that can be considered as different cell states, since the cells in mitosis respond differently than cells in G1 phase to the same stimuli. More important for this review are the clinically important states of transformation in cancer (perhaps related to wound healing) that is most common in the keratinocytes because of their continual growth and senescence in aging that is most evident in skin fibroblasts. Our studies of transformed cells indicate that there are major changes in the cell mechanical properties upon transformation such as the loss of rigidity sensing and transformed cells share some of the expected changes in wound healing and regeneration [1]. Although we understand less about aging and senescence, the preliminary studies of primary skin fibroblasts from aged donors indicate that they have dramatically different mechanical properties than those from younger donors [2-6]. The concept of cell states in the context of cancer and aging is useful in that transformed and senescent cells from many different tissues share common mechanical and biochemical properties [7]. For example, we have found that tumor cells from many different organs and at different cancer stages can all be killed by mechanical perturbations [8, 9], which can open new paths for treatment. Thus, I suggest that common features of transformation and of senescence can help to elucidate biochemical and mechanical treatments to counteract the negative effects of those states in many different tissues.

Skin Inflammation and Skin Tumor Cells Involve Common Players

It is commonly believed that repeated wounding and inflammation in a tissue increases the risk of cancer, e.g., sun exposure and skin cancer are strongly correlated. Not surprisingly, wound healing and cancer have many common players at a biochemical level. For example, a major risk factor in tumors is the upregulation of microRNA 21[10-12] and a screen of 169 cancer-associated microRNAs found that Mir-21 was best in promoting Squamous Cell Carcinoma tumor growth[13]. As predicted, Mir-21 is upregulated in wound healing of skin, many other tissues and even in limb regeneration of salamanders[14-17]. Because wound healing involves the stimulation of growth of adult cells, there can be long-lasting growth of cells when they don't revert to a normal phenotype after the repair is complete (Figure 1). This unregulated growth state normally does not produce large tumors because of the many limitations on excessive cell growth that are present. However, with cancer progression some unregulated growing cells become immortalized, change their metabolism and acquire characteristics of serious cancers. Thus, we show in the figure that many other factors appear with cancer progression (Figure 1, Box C) and are aided by local growth signals (Figure 1, Box B); however, a common feature of all tumor cells is the ability to grow (Figure 1, Box A). By understanding the basis of the loss of control of growth, it may be possible to design therapies to target those cells specifically.

From the early days of cancer studies, growth of tumor cells on soft agar was a defining feature, since normal cells would die on the same substrates. We now have strong evidence that the loss of rigidity sensing allows growth on soft agar and restoration of rigidity sensing in tumor cells blocks growth on soft agar as well as the tumor phenotype[7]. Like complex machines, cell rigidity sensors can be depleted by depletion of any one of many proteins involved in their function. For example, the cytoskeletal protein, tropomyosin 2.1, is required for rigidity sensor function and is depleted in many cancers. Restoration of tropomyosin 2.1 levels in those tumor cells causes normal cell behavior (blocks tumor growth and metastasis) and depletion of it in primary fibroblasts causes transformed growth on soft agar. By modulating the levels of just tropomyosin 2.1, the cells can change from tumor growth to normal growth states. This major change in cell state is reflected in the changes in levels of over 700 mRNAs when rigidity sensors are restored in tumor cells or depleted in fibroblasts [7]. Thus, if we can find features of a majority of transformed cells that are different from normal cells, then we can potentially exploit those differences to inhibit the growth of many different types of tumor cells.

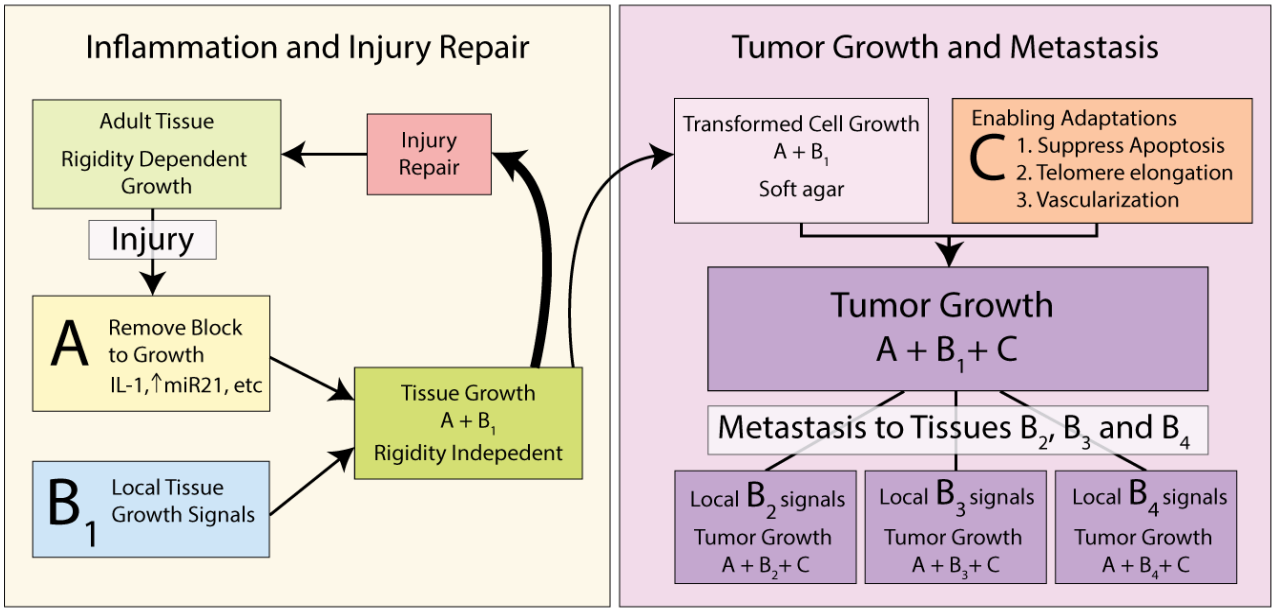

Figure 1. This diagram outlines the basis of tumor growth as a result of repeated inflammation and injury. When injury occurs, the blocks to normal cell growth are removed to enable cell growth (A). This appears to involve the depletion of rigidity sensors that enables the cells to grow on many different surfaces[7]. Growth to heal the wound also involves local growth stimuli (B_1), including hormones and mechanical stimuli. Once the blocks to growth of adult cells are removed for healing, there is a low but significant probability that they will not be restored during injury repair, resulting in tumor growth, i.e. the transition to tumor growth and metastasis. However, additional adaptations (C) are needed to produce major tumors that can metastasize and form new tumors in different environments with other growth stimuli (B_2-B_4). However, it is the absence of the block to growth (A) in the transformed state that is common to all tumor cells and features of transformed cells can be targeted to inhibit the growth of many different tumors.

Surprisingly, transformed cells are mechanosensitive and will undergo apoptosis after cyclic stretching or low-level ultrasound exposure. Skin unlike many other tissues is easily stretched and skin tumors may be sensitive to cyclic stretching. Cyclic strains of 3-7% with a period of about 1 Hz are sufficient to cause apoptosis of many tumor cells after hours of treatment including melanoma cells [8]. In the limited experience that we have had with mechanical activation of apoptosis in tumor cells, there is a relatively narrow window of treatment parameters that will cause specific death of tumor cells. Greater deformations can damage neighboring normal cells and the specific architecture of tumors may shield the tumor cells from significant deformations making the treatment ineffective.

The process of apoptosis upon stretch of tumor cells is different than the process of apoptosis of normal cells on soft substrates and involves a calpain-dependent activation of caspases after mitochondrial damage. An endoplasmic reticulum-mitochondrial stress pathway is similar to the pathway activated by stretch of tumor cells and other studies indicate that tumor cells are particularly sensitive to activation of calpain-dependent apoptosis[18]. In more recent studies, we find that the mechanical effects of low frequency ultrasound mimic stretching in terms of activating the apoptosis of tumor cells[9].

The periodic stretching of cells is particularly relevant to skin which is constantly experiencing strains from the environment and can be beneficial in many ways. External strains can come in many different forms and interpreting how they are affecting cell functions requires a standardized perturbation. There are often variable results of experiments on effects of stretch or massage in humans because different people will do things differently. Similarly, timing is an important parameter and the frequency of mechanical stresses in massage devices that are designed to strengthen skin appears to matter (https://www.cosmeticsbusiness.com/news/article_page/LOreal_reveals_first-look_at_new_Clarisonic_massage_device/127736). More highly controlled experiments are needed to have a better understanding of the short-to-medium term effects of external strains on skin.

Aging of Skin and Senescent Cells

As we age, there are major changes in skin that are being addressed by many cosmetic companies as well as biomedical researchers. From an evolutionary viewpoint, a species is better able to adapt to environmental challenges if individuals die after their peak reproductive age. Thus, aging and death are programmed, and the issue is how to maintain a good quality of life while delaying the inevitable. One hallmark of aging is cell senescence, which is a cell state like the transformed cell state in that senescence can occur in cells from many different tissues. Senescent cells

are characterized by a lack of growth, increase in cell size, and the senescence associated secretory phenotype (SASP) which is a complex cocktail of proteins secreted by senescent cells that can induce senescence in neighboring cells. Aged skin fibroblasts produce a similar cocktail of inhibitory molecules that appear to be skin specific[6]. It is expected that a negative feedback cycle can occur with increased levels of senescent cells causing further senescence. One strategy to combat aging is to kill the senescent cells with senolytic treatments to cause apoptosis of senescent cells [19]. There appear to be benefits to the killing of the senescent cells but the treatments have not reached the clinics [20].

The biochemical basis of the senescent phenotype is not known but there are dramatic changes in mitochondrial fusion with senescence that indicate that increased fusion of mitochondria will cause senescence[21]. Thus, in both the transformed and senescent cell states, mitochondria and their mechanical properties appear to play prominent, although different roles.

In the case of aging skin, the quasi-steady state of skin thickness and tension changes with time and aging. With the constant turnover of skin cells every two to four weeks, there is the chance for remodeling to accommodate the changes in the dermis. The decrease in activity with age favors senescence of the fibroblasts; however, the magnitude of the strain with mechanical activity has important effects. In the case of normal skin fibroblasts, periodic strains of 20% can inhibit growth [22] whereas periodic strains of 3-7% can stimulate growth of primary fibroblasts [23]. Further, in the case of aged skin fibroblasts, preliminary studies (Cui et al., unpublished results) indicate that 10% periodic strains cause a dramatic increase in cell area relative to young fibroblasts. This highlights the need to analyze a range of parameters for the cells of interest to know what level of physical activity can be beneficial.

Exercise has Anti-cancer as well as Anti-aging Effects

Physical strain of tissues through exercise can cause a number of biochemical changes in the body that are beneficial. Although we don't really understand how strain is transformed into beneficial biochemical signals, there is an increasing body of evidence indicating that regular exercise results in better health than inactivity. In the case of cancer, there are many studies that have found a strong correlation between exercise and an inhibition of cancer (National Cancer Institute lists 7 cancers where exercise inhibits the disease and another 8 where preliminary evidence exists, (https://www.cancer.gov/about-cancer/causes-prevention/risk/obesity/physical-activity-fact-sheet). One way to explain this correlation is through the fact that physical activity strains tissues and could thereby cause tumor cell apoptosis; however, there are hormonal effects of exercise that also are beneficial. In the case of senescence, there is significant research support for the old adage of "use it or lose it" since epidemiological studies of people who regularly exercise compared with those who don't, show definite benefits of exercise. Further, evidence is mounting that exercise itself is senolytic and causes the loss or death of senescent cells as well as stimulating the growth of normal cells[24]. Our early studies of low frequency ultrasound indicate that it will kill tumor cells and we are testing effects on senescent cells. Cancer occurs because of the loss of mechanosensing of rigidity and senescent cells have decreased responses to mechanical perturbations. Both cell states exhibit altered mitochondrial behavior that belies altered metabolism. In the future, it will be important to better understand how physical activities or physical interventions can be employed to mitigate the negative effects of cancer and aging.

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MPS has financial interests in Mechanobiologics, Inc. that is developing and testing ultrasound-based therapies for cancer and aging. Clinical trials are under way.

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