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REVIEW ARTICLE

The axolotl model for cancer research: a mini-review

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Summary

The Mexican axolotl (Ambystoma mexicanum) is one of the most widely used laboratory animals for research. It is able to regenerate multiple structures including the limbs, jaws, tail, spinal cord and skin among other organs. The mechanisms governing regeneration, wound healing, development, and cancer formation are closely linked. There is increasing evidence highlighting the common signalling pathways which link to cancer growth and regeneration whereby dysregulation of the well-balanced and coordinated process of regeneration leads to cancer. This review aims to highlight the regenerative capacity of axolotls and identify how the active molecules from regeneration extracts could lead to major benefits, with directions on how to develop therapeutic approaches for cancer treatment in humans.

Key words: *ambystoma mexicanum, regeneration, de-differentiation, re-differentiation, cancer*

Introduction

Salamanders are one of the oldest laboratory animal models used for research experiments. In 1768, Lazzaro Spallanzani first described limb regeneration in salamanders [1,2]. One of the earliest regenerative animals that made it to Europe all the way from Mexico is the *Ambystoma mexicanum*, more commonly known as the axolotl. It belongs to the class *Amphibia* family: *Ambystomatidae*. Described as larva, they were later classified as adults when it was observed they were able to reproduce. The axolotl became a very important model in the field of embryology and developmental biology [3], as well as research on thyroxine. Nowadays, the axolotl plays an important role in investigating and understanding stem cell biology [4], cancer [5], scarless wound healing, and aging [6].

What makes axolotls ideal models for research in cancer treatment?

Several types of organisms such as hydra, planarians, echinoderms, annelids, and amphibians can completely regenerate several tissues, organs or even whole body parts. Regeneration is usually classified as morphallaxis or epimorphosis. In morphallactic regeneration, such as in hydra, the remaining part of the amputated organism is remodelled to regenerate all parts of the body. In contrast, epimorphic regeneration, as seen in urodele amphibians, involves the regeneration of various tissues and complex structures. The axolotl and the newt (*Notophtalmus viridescence*) are the two most commonly used urodele amphibians in the laboratory and they are capable of regenerating different organs. The fact that they are able to regenerate complex structures makes the urodele amphibians important animal models not only in studying and understanding how regeneration occurs but also in the study of cancer.

The axolotl is one of the few tetrapods which are capable of regenerating complex biological structures such as limbs, tail, heart, eye lens, and central nervous system including the brain and

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spinal cord [7]. Earlier research on the effect of carcinogens on axolotls has shown that they are resistant to tumour formation upon exposure to carcinogens [8]. In other experiments, tumor cells showed regression or they were incorporated into the normal tissues of the organism [8].

The axolotl limb regeneration model is amongst the most studied regeneration models and the fact that many genes and signalling pathways are conserved between humans and axolotls makes this organism an ideal model in therapeutic research. In fact, even though the axolotl genome is 10 times larger than the human genome at 32 gigabases (GB), it roughly encodes a similar number of proteins as humans [1] with extensive conservation of synteny (blocks of order within two sets of chromosomes that are being compared with each other) between *Ambystoma*, chicken, and human, and a positive correlation between the length of conserved segments and genome size [9]. Several other reasons make the axolotls very attractive models for research, including their high reproductive rate and low maintenance requirements in the laboratory. In comparison to the newt (*Notophtalmus viridescence*), axolotls are much easier to breed. The fact that axolotls are sexually mature and yet maintain a larval state could prove to be pivotal to cancer research.

Regeneration and Cancer

Regeneration is a homeostatic process of renewal that involves a well-balanced and coordinated process of restoration of cells, tissues, and organs that have been damaged or lost, resulting in total recovery of the structural and functional integrity of the part involved. It is characterised by tightly controlled and orderly overlapping processes of inflammation, tissue reconstruction, and remodelling [10]. The process of regeneration must also integrate the newly-formed tissues with the pre-existing tissues. Moreover, regeneration must be able to have an orderly control to initiate and maintain signals, with the capacity to regulate the cell cycle, resulting in a finite number of cells that undergo division. Once the regeneration is complete, the process must be terminated. A failure to switch off the signals that govern proliferation would lead to abnormal tissue repair, fibrosis, and uncontrolled cell growth or cancer [11].

In humans, the ability to replace cells within a specific tissue is limited. With a few exceptions such as the finger tips, involving the distal/terminal phalanges, and the liver, humans are unable to regenerate more complex structures that integrate the structure and function of multiple tissues [12]. Even though humans, to some extent, can repair tissues such as bone, peripheral nerves, and skin cells following ultraviolet exposure, these mechanisms are not as efficient and error-free as the regenerative abilities of certain animals. It is important to note that even though these organisms are far superior in their regenerative capacities when compared to humans, the signalling pathways that govern regeneration, embryonic development, wound healing, and cancer are highly conserved in both humans and organisms with regenerative capabilities [13]. In fact, epithelial-mesenchymal transition (EMT), which is central for embryonic development, is also important during wound healing and regeneration and if unregulated, can lead to cancer. The signalling pathways (*Notch, hedgehog (HH), Wnt, RTK, and TGFß*) that regulate EMT are common among regeneration, embryonic development, wound healing, and cancer.

In 1935, Waddington postulated the existence of 'individuation fields', agents which control the growth of different parts of an organism in a harmonious way so that a normal individual is formed [14]. Cancers may form following an escape of cancer cells from the controlling influence of an individuation field and these cells might be brought under control again if they were exposed to the influence of a particularly strong individuation field, e.g. the regeneration field of a regenerating urodele limb [15]. This theory might be supported by the observation that spontaneous tumours are rarely observed in animals which are capable of regeneration. However, it cannot be true for all regenerating organisms since the reactions of planaria and axolotls to carcinogen exposure are different. Whereas axolotls resisted to cancer formation, exposure of planaria to carcinogens induced tumour formation [8, 16]. In fact, the tumour microenvironment plays a pivotal role in carcinogenesis, whereby there is direct interaction between the tumour and the microenvironment in which it resides.

Axolotls: Regeneration and Cancer

Following limb injury in axolotls, regeneration is initiated and the basal membrane keratinocytes migrate as a sheet to cover the wound site. They proliferate to form the wound epidermis and within days the wound becomes innervated, forming the apical epithelium cap (AEC). The contact between the AEC and the underlying cells from the remaining limb help determine the orientation of the regenerating limb. The AEC provides pro-regenerative signalling molecules, including *FGFs, BMPs, TGFß, IGFs* and *WNTs*, which are known to be essential for limb outgrowth during development and adult salamander regeneration. The interaction between the AEC and the underlying tis-

sue also results in the formation of the blastema, which is structurally and functionally equivalent to a limb bud in the embryo. During the early stages of regeneration, the AEC supports blastema formation by promoting cellular dedifferentiation, which consists of cell cycle re-entry of post-mitotic differentiated cells [17]. The blastema is likely to be composed of both dedifferentiated cells derived from muscle cells, dermal fibroblasts, as well as activated stem/progenitor cells.

Dedifferentiation occurs together with protease-induced histolysis and the release of cells from their tissue matrix. Proteomic studies have identified a number of genes that are associated with dedifferentiation, including *msx, Nrad, rfrng* and *notch*. Even though blastema cells are not reprogrammed to pluripotency, they express three of the four transcription factor genes used to reprogram adult somatic cells to pluripotency, namely, *Klf4, Sox 2,* and *c-myc* [18]. Changes in chromatin-associated proteins were detected, suggesting transcriptional changes, chromatin modification, and upregulation of tumour suppressors such as the Kruppel-like factor 6, and Sox 6, which is required for neuronal and skeletal differentiation. Proteins from blastema-enriched transcripts, *cirbp* and *kazald1*, have been particularly implicated in blastema formation [19]. Other proteins implicated in extracellular matrix structure and synthesis (*ugdh, slc23d2*), regulation of EMT (*fam3c, hmga2*), and regulation of epidermal cell differentiation and proliferation (*tgm1, ovol2, lmo7, ehf, ereg, sorbs3, eppk1*) have also been identified [18]

Successful regeneration will only proceed if none of the above-steps are impeded. Also, signalling above a threshold level from the nerve is necessary, for both initial blastema formation and growth and development of the blastema during the early and mid-stages of regeneration. In addition, rather than the type of nerve, it is the quantity of damaged nerves that is important in regeneration. Also, denervation or diversion of the nerve at different stages of blastema formation, or macrophage depletion leads to impaired communication between the epidermis and underlying cells and regeneration failure [20].

Similar crosstalk between cancer cells and neuronal cells has been observed, whereby a reciprocal interaction results in neuronal outgrowth by cancer cells and induction of cancer metastasis by neuronal cells [21]. Involvement of nerves in tumour growth and metastasis has been described for several tumours, including basal cell carcinoma [22], gastric [23] and prostate cancers [24]. It appears that neurotransmitters released by nerves and proteins such as BMP2 and FGFs have a direct effect on both regeneration and cancer microenvironments.

The potential of axolotl research in cancer treatment

From a cancer perspective, the ability of axolotls to faithfully replicate regeneration following injury including dedifferentiation of cells, gain of proliferative capabilities, and the subsequent re-differentiation without producing abnormalities is of great relevance. Additionally, the limb regeneration models provide an important research tool to dissect the pathways that enable regeneration-capable animals to evade cancer formation.

To aid in identifying any active molecules, factors or proteins that can possibly cause differentiation of cancer cells, it is important to focus on secretary proteins including factors secreted within the extracellular matrix and that can have a direct effect on the tumour microenvironment. It is equally important to dissect in detail the process of regeneration and characterize the different factors involved in regeneration within the context of cancer treatment. The ability to identify the factors that cause re-differentiation will be helpful in cancer treatment. Such factors are important because they act on early progenitors (blastema) and result in differentiation into the more complex structures that eventually replace the lost limb. Molecular characterization of regeneration will help identify and isolate the factors involved in re-differentiation and avoid the adverse or deleterious effects that factors can have on cancer cells. Success of such therapeutic approaches depends on whether the molecules or factors can actually be taken up by ligands or cell surface receptors and whether they have a differentiation, apoptotic, and/or cytotoxic effect on cancer and normal cells.

Since some cancers are caused by a block in differentiation, addition of a protein extract from the regenerating limb of axolotls could theoretically cause the followings: activation of differentiation pathways without necessarily correcting the underlying insult that initiated the differentiation block; rectification of the initial defect causing the differentiation blockage; and/or cause epigenetic modifications resulting in a more open DNA state which may expose transcription sites allowing access to other differentiation, causing drugs like all-*trans* retinoic acid (ATRA). As a therapeutic strategy, differentiation therapy can induce tumour differentiation or growth arrest by reactivating the normal control mechanisms that regulate cell proliferation, which is also a less toxic approach to cancer therapy when compared to the more cytotoxic chemotherapeutic drugs currently used to treat cancer patients. Results of studies

on cell lines and animal models that aimed at inducing malignant cells to overcome their block of differentiation and enter the apoptotic pathways as an elegant alternative to killing cancer cells by cytotoxic therapies have been described [25]. Substances such as phorbol diesters, teleocidins, polar planar drugs, cytokines, retinoids, and vitamin D metabolites showed promising results when tested on different leukaemia cell lines [25]. The classic successful story of differentiation therapy is the use of ATRA for the treatment of acute promyelocytic leukaemia (APL). ATRA in combination with arsenic trioxide has become the standard treatment for APL, resulting in cure rates up to 90% [26].

With the advent of molecular biology, the focus is on the identification of the genes, transcription factors and signalling pathways orchestrating the cellular events that govern regeneration and cancer. In addition, the potential benefit that this field of research offers for the eventual development of novel medical treatments is of great importance. Also, new technologies such as CRISPR, RNA-seq, together with established molecular approaches such as lineage tracing, transplant assays, and *in vitro* modelling have been extremely successful in identifying new interacting proteins in governing pathways, further allowing understanding pathway modulation and dynamics. Although much is known about the mechanisms of regeneration in the axolotl, the fine lines between controlled and uncontrolled cellular proliferation as seen in regeneration and cancer are not fully understood. From this perspective, studying the overlapping stages of regeneration and focusing on the factors and/or molecules that cause re-differentiation becomes very important.

There are huge challenges in designing drugs that not only target the cancer cells but can also modulate the microenvironment to one that does not favour the cancer cell phenotype. By understanding the processes governing cellular proliferation, de-differentiation, cell cycle arrest, and re-differentiation using animal models like the axolotls, further light may be shed on the pathways linking regeneration and cancer with the ultimate aim of discovering novel therapeutic approaches in the treatment of cancer.

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Conflict of interests

The authors declare no conflict of interests.

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