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Review

Regeneration: The origin of cancer or a possible cure?

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ABSTRACT

A better understanding of the forces controlling cell growth will be essential for developing effective therapies in regenerative medicine and cancer. Historically, the literature has linked cancer and tissue regeneration—proposing regeneration as both the source of cancer and a method to inhibit tumorigenesis. This review discusses two powerful regeneration models, the vertebrate urodele amphibians and invertebrate planarians, in light of cancer regulation. Urodele limb and eye lens regeneration is described, as well as the planarian's emergence as a molecular and genetic model system in which recent insights begin to molecularly dissect cancer and regeneration in adult tissues.

Contents

> *"The individuation field, then, is the agent which controls the growth of the different parts in a harmonious way so that a normal individual is formed. In later life, the individuation field splits up into smaller separate fields, such as leg fields, head fields, etc. These are the agents from which cancerous growth has escaped."*
> (Conrad H. Waddington, 1935).

1. Introduction

In his 1935 treatise, Waddington considers the mechanistic connection between uncontrolled cancerous growth and controlled embryonic development, postulating the existence of "individuation fields" that regulate tissue growth both during embryonic development and in adult tissues [1]. Interestingly, Waddington's description of "individuation fields" is evocative of regenerative fields, and he himself links a field's strength to the organism's regenerative ability. Modern interpretation of Waddington's theory, which remains untested and largely overlooked in the current literature [2], implicates regeneration mechanisms as possible cancer regulators and underscores the need to investigate links between regeneration and cancer.

The term regeneration implies a well-coordinated restoration of cells, tissues, and organs that have been physically or functionally lost. This reparative process must accomplish the recognition and recapitulation of missing structures, while simultaneously achieving functional integration between recently formed and pre-existing tissues, in order to direct physiological and structural alterations. Furthermore, regeneration involving cellular proliferation (epimorphosis [3]) requires instructive signals with the capacity to efficiently regulate cell cycle, resulting in a finite number of cells that undergo division and complete repairs [4–7]. Participating cells must be precisely guided to needed areas, and once regeneration is complete specific cues are required to report

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regenerative success and signal termination. Otherwise, the initial response would continue indefinitely, causing undesirable consequences for body homeostasis.

Diverse regenerative phenomena appear to utilize similar mechanistic procedures, including: cellular replacement (e.g., physiological cell turnover), local tissue repair (e.g., epithelial wound repair), and regeneration of large sections (e.g., appendages and head) [4,7–12]. Independent of magnitude, a regenerative event always seeks to maintain or reestablish both form and function (morphostasis). However, the process is not infallible, as demonstrated by growing evidence associating regeneration with cancer-related cellular abnormalities [1,8,10,13–19]. Owing to space limitations, this review will be restricted to analyses of the relationship of abnormal cell proliferation and cancer to injury-induced epimorphic regeneration in adult animals. Readers interested in the relationships between cell turnover and regeneration are referred to a recent review [9]. Despite extensive accumulated data on epimorphic regeneration (both blastemal and non-blastemal [6]) consistently linking regeneration and cancer, we still lack a mechanistic connection [1,8,10,13–18,20–23].

Seemingly contradictory, regeneration might in fact both contribute to the source of abnormal growth and also provide a means to prevent and correct growth abnormalities. The initial phenomenological observations were summarized by Seilern-Aspang and Kratochwil, who surveyed the classical data and proposed two nonexclusive hypotheses: (i) the formation of malignant tumors derives from an impaired or incomplete regenerative process (Fig. 1A), and (ii) the regeneration process may bring under control the autonomous growth of malignant cells (Fig. 1B) [16]. The first hypothesis is largely based on observations of local tissue repair in mammals, where epithelial surfaces exposed to chronic damage or hypoxic conditions and inflammation result in growth aberrations during the regenerative response [8,10,13,15,16,19,23,24]. It is important to note this is not universal to all cancers but is perhaps more likely in those of epithelial origin. Interestingly, alterations of this process are probably associated with loss of tissue formation, remodeling (morphogenesis), and termination signals, whereas the capacity to sense damage and activate cell proliferation may not in fact be impaired. This suggests characterizing the signals associated with later regeneration events may be useful in identifying candidates that halt abnormal cell proliferation (leading to cancer). The second hypothesis conversely suggests that if induced cellular proliferation during regeneration is followed by morphogenetic processes, regeneration has the potential to prevent abnormal growth and, more startling, to reverse malignancies and regain morphostasis—phenomena mostly observed in animals with immense regenerative potentials [1,14,16,17,21,25–27]. It is intriguing that biological responses associated with epimorphic regeneration lead to completely opposite outcomes—in one instance destruction, while in another rebuilding and reestablishing form and function. It is the latter of these outcomes that is the major concern of this review.

Cancers have been regarded as wounds that never heal, and the possible relationships between carcinogenesis, inflammation and local tissue repair (wound healing) have been extensively reviewed elsewhere [8,10,13,19,24]. This article will focus on two adult animal models, the vertebrate urodeles and the invertebrate planarians, that have been traditionally used to study the relationships between large-scale regeneration and malignant transformation. In addition, we will briefly survey some of the approaches and data (mostly classical) concerning cancer and the different regeneration methods in urodele amphibians and planarians. Finally, recent molecular approaches targeting evolutionarily conserved signaling pathways in planarians will be discussed, highlighting the relevant

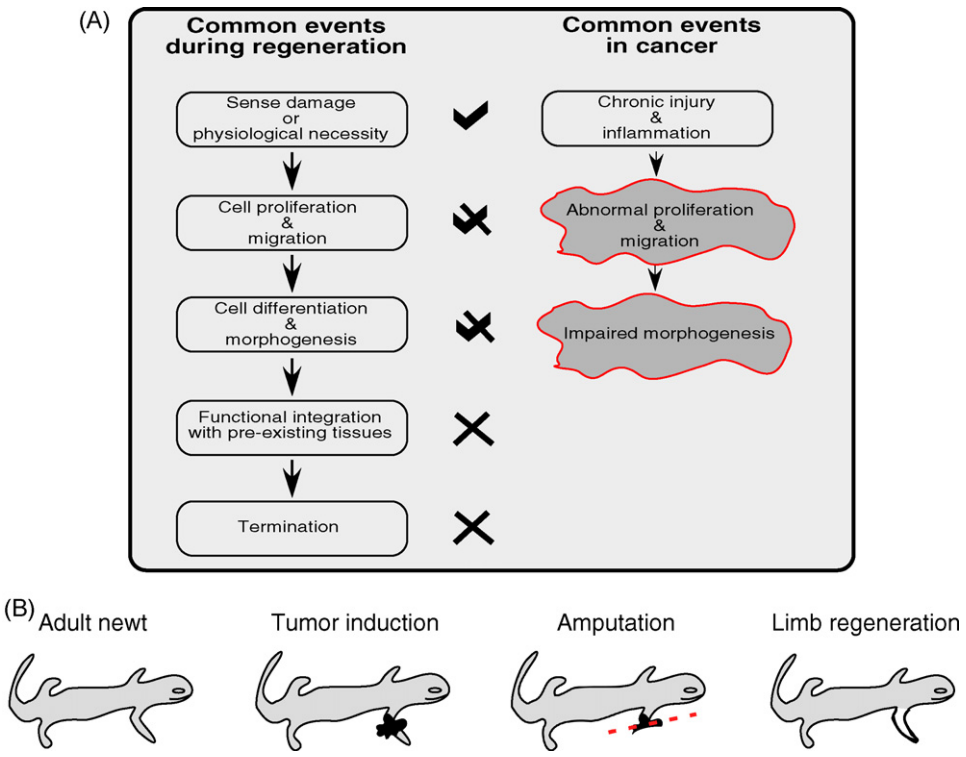

Fig. 1. Links between regeneration and cancer. (A) Regenerative events and their corollaries in cancer. Importantly, the process of regeneration can be repeated without causing malignant transformation, while in cancer the regenerative process is incomplete such that chronic injury and inflammation leads to continuous proliferation. This suggests that characterizing signals at later stages of regeneration (especially those involved in termination) may help identify candidates able to stop abnormal proliferative responses to chronic injury. (B) Regeneration can correct malignant transformation, as in newt limbs where amputation through the site of induced tumors results in the regeneration of a normal limb without tumors ([16,21,57] and references therein).

consequences that may serve as an entry point for unraveling the molecular bases linking cancer and regeneration in adult organisms.

2. Urodeles and planarians as models for studying regeneration and cancer

Although many different model animals, developmental stages, and *in vitro* systems have been utilized to study cancer and regeneration independently, two organisms, the urodele amphibians (including newts and axolotl) and planarians (flatworms), have risen to the forefront for simultaneously exploring malignant transformation and adult regeneration [1,14,17,25,26,28,29]. The interest in these two particular groups comes from their extraordinary regenerative capacities, which have been explored for over 200 years [4–6,11,22,30–35], as well as their distinct capacities for chemically induced carcinogenesis [14,17,26,29,36–38]. Remarkably, even though urodeles and planarians both undergo epimorphic regeneration, the cellular mechanisms they use to repair missing parts and their sensitivities to carcinogens differ.

For example, in adult newts injury causes differentiated postmitotic cells to re-enter the cell cycle and dedifferentiate, while in planarians the main regeneration strategy involves proliferation of resident adult somatic stem cells known as neoblasts [5,11,31,33–35,39]. Fascinatingly, while regenerative tissues in urodele amphibians display a low frequency of tumor development upon carcinogenic exposure [14,16,17,40], treatment of whole planarians with similar chemicals can lead to abnormal proliferation and tumor formation [16,20,29,36–38,41,42]. Given the astonishing regenerative capacities of both planarians and urodeles, their differential responses to carcinogens offer a unique opportunity to examine Waddington's theory that "individuation fields" controlling growth are the source from which "cancerous growth has escaped" [1].

These two models of epimorphic regeneration evoke a provocative scenario where well-orchestrated molecular events shape growth and morphogenesis in adult tissues by preventing random growth aberrations during the reversal of differentiated states (urodele) and abnormal stem cell proliferation during *de novo* tissue formation (planarians). Most of our current knowledge regarding cancer and regeneration in both amphibians and planarians comes from classical studies using chemically induced carcinogenesis. In these studies, treatment with substances known to produce permanent DNA alterations was used, followed by histological and behavioral analyses to evaluate effects. These data consist mostly of phenomenological observations lacking the resolution achievable with modern molecular and genetic techniques. The following sections provide an overview of our current understandings about the specific regenerative properties and cancer associations for each model system.

3. Urodele amphibians and cancer

Adult newts and axolotls possess amazing regenerative potential. The newt, for example, can regenerate tail, limbs, brain, spinal cord, retina, and lens [4,5,11,22,30,39]. Although these vertebrates regenerate multiple tissue types, the analyses here are restricted to regeneration of adult newt limbs and lens because these tissues have been more extensively studied and related to cancer. The processes of lens and limb regeneration demonstrate an incredible degree of plasticity, where both differentiated pigment epithelial (PE) cells from the dorsal iris as well as limb mesenchyme reverse their differentiated state by re-entering the cell cycle. In both cases, this cellular dedifferentiation implies a loss of tissue-specific characteristics (bringing cells close to a undifferentiated state) followed by re-differentiation into cells of the same type or even a different lineage [30]. More importantly, this process can be repeated over and over without variation—a feature apparently missing in mammalian models where chronic local wound repair can lead to certain epithelial cancers [8,13,15]. Nonetheless, while both newt tissues undergo epimorphic regeneration, the processes are sufficiently distinct that sub-classifications of regeneration have been proposed [6]. In lens regeneration cellular dedifferentiation, transdifferentiation (epithelial cells to lens), and proliferation are observed [11,14,43], while in limb regeneration dedifferentiated cells participate in the formation of the regeneration blastema (basically a mesenchymal growth zone where missing parts are rebuilt) [4,5,11,14,22,39,44,45]. Thus, adult tissue regeneration in urodeles provides a useful setting where transdifferentiation and blastema formation can be analyzed to elucidate how following injury cells are able to reverse their differentiated state and proliferate and yet escape the pitfalls of malignant transformation.

3.1. Lens regeneration and cancer

Shortly after lens removal, dedifferentiated PE cells of the dorsal (but not ventral) iris lose their pigmentation and begin to elongate, differentiating into primary lens fiber cells; cell proliferation and crystallin synthesis follows, rebuilding the missing lens in about 25 days [11,14,43]. Lens regeneration in adult newts provides an exquisite model for studying cellular plasticity, because within the same organ structurally similar components display fundamental differences in regeneration potentials—while the dorsal iris is a source for regeneration, the seemingly similar ventral iris is not. Only a few studies have addressed the possible application of urodele lens regeneration to cancer research. In most of these studies, potent carcinogenic compounds such as nickel subsulfide, known to induce chromosomal abnormalities and DNA strand breaks (and in other vertebrates, abnormal outgrowth and cancers), were used as a means to evaluate cancer and regeneration simultaneously [17,26,46–50].

In the majority of these studies the carcinogenic compounds were introduced locally within the eye, and the consequences of such treatments during lens regeneration can be summarized as follows (Fig. 2A): (i) regeneration proceeded normally compared to untreated animals, (ii) supernumerary lenses arose from the *ventral* iris without additional abnormalities, or (iii) in some cases there was a delay of several months (or complete inhibition) of regeneration accompanied by production of melanoma-like ocular tumors originating exclusively from the ventral iris. These results strongly support the belief that regenerative tissue is significantly unlikely to form cancerous abnormalities. This is particularly well illustrated in the newt lens, since the dorsal iris (capable of regenerating lens) does not act as a source of abnormal cells, while its non-regenerating counterpart (the ventral iris) responds to carcinogenic insults by forming abnormal cells that give rise to tumors.

Under these conditions, the dorsal iris is basically resistant to tumor formation without any effect on its regenerative response. Even when exposed to higher concentrations of carcinogenic compounds, the dorsal iris simply stops regenerating (still without forming tumors). Conversely, in the ventral iris these chemicals have the capacity to unlock a highly uncoordinated regenerative potential that can produce supernumerary lenses or even malignant transformations leading to aggressive tumors. Therefore, the differences between the dorsal and ventral iris are not restricted to their regenerative potentials, but also include their abilities to respond to cancer-inducing cues, suggesting a link between regeneration and cancer avoidance. Interestingly, this can be analyzed simultaneously *in vivo*, as has been previously shown [26]. Thus, the lens regeneration paradigm provides an entry point for dissecting molecular differences between regenerative and non-regenerative

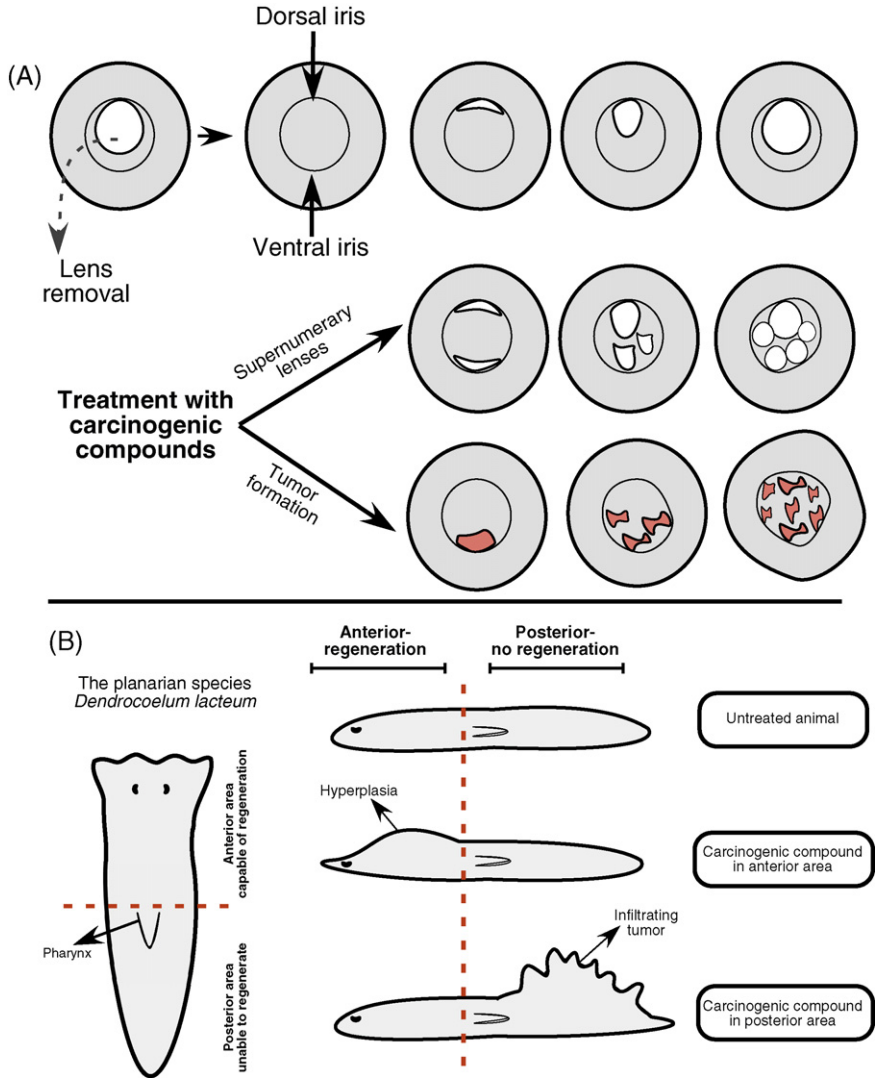

Fig. 2. Differential carcinogenic responses in regenerating versus non-regenerating tissues. (A) Vertebrates. Newt dorsal iris regenerates while ventral iris does not. Lens removal and dorsal iris regeneration into lens (top row, adapted from [14]). Treatment with carcinogens causes (second row) supernumerary lenses to arise from the ventral iris [50], or (third row) inhibition of lens regeneration and ventral iris-derived tumors (light red) [26]. (B) Invertebrates. In one planarian with limited regenerative abilities (*Dendrocoelum lacteum*), anterior tissue regenerates while posterior tissue does not. Anterior treatment with carcinogens causes mild differentiated hyperplasia but posterior exposure induces infiltrating tumors (adapted from [16]). Remarkably, decapitation of worms with posterior tumors leads to tumorigenic tissue differentiating into accessory pharynx, suggesting that long-range signaling during regeneration could modulate the behavior of aberrant cells [16]. In both cases (A and B), regenerative tissue is more resistant to carcinogenesis than non-regenerating tissue.

tissues [51], as well as the opportunity to investigate Waddington's "individuation fields" and their differential capabilities in regenerative versus non-regenerating tissues [1].

3.2. Limb regeneration and cancer

There is an impressive amount of literature associated with limb regeneration in urodeles. While this is arguably the most studied subject in vertebrate appendage regeneration, the molecular mechanisms remain elusive. Recently, several molecular and genomic tools (e.g., microarray, *in situ* hybridization, loss of function, transgenesis) have been developed to analyze limb regeneration, offering greater opportunities to increase our understanding [4,5,30,32,45,52,53]. As with lens regeneration, differentiated post-mitotic limb mesenchymal cells dedifferentiate upon injury and re-enter the cell cycle. However, limbs differ from the lens in the formation of blastemas. Dedifferentiated cells in the limb migrate and extensively proliferate, building up a regeneration blastema in which cells eventually re-differentiate to functionally reestablish missing parts. Under normal conditions, these sequential events can be recapitulated numerous times without adverse effects—indicating the presence of well-established regulatory signals seemingly unaltered by repetitive damage. Moreover, amputation at different points along the proximal–distal axis in each case specifically recreates the missing parts, demonstrating the newt limb's great ability to integrate positional information with injury/repair mechanisms [4,5,11,22,31,32,44,45,53].

From a cancer point of view, it is intriguing that adult tissues are able to reverse differentiated, post-mitotic cells to progenitor stages, thereby gaining migratory capabilities and massive proliferative properties that enable subsequent (re)differentiation, all without producing abnormal cell growth. The urodele limb model supplies a tantalizing example of how differentiated cells can be transformed into progenitor-like states still developmentally controlled and able to form multiple, functional tissues. (This phenomenon has been extensively investigated both *in vivo* and *in vitro* and is discussed elsewhere [5,11,14,22,31,32,39,44,45,53].) Additionally, the limb regeneration paradigm also provides a

convenient context for learning about the intrinsic ways in which damaged adult tissues evade the biological mistakes leading to cancer-related abnormalities. The importance of this is underappreciated in the modern literature, where the existing data are mostly from older studies. Historical attempts to uncover how urodele limbs retain developmental plasticity but avoid cancer were mainly based on the local application of chemical carcinogens [16,17]. Many of the results from these approaches were somewhat controversial and difficult to interpret, but mostly they concluded that limb-regenerating tissue in urodele amphibians displays a very low rate of malignant tumor formation, such that these animals are commonly regarded as tumor resistant [14,17]. This is not strictly a urodele phenomenon, since another vertebrate model has displayed similar tumor resistance [54]. However, it must be noted that resistance does not equate to an inability to form tumors.

Similarly to lens regeneration, local treatment of the regenerating limb with carcinogens results in altered regeneration patterns without inducing tumors, suggesting that some of these chemicals may modify the tightly regulated conditions required for efficient tissue repair [55]. However, under certain conditions, malignant transformations (e.g., from the mucous glands of the skin) can be induced in newts after chemical treatment, confirmed by histological and pathological analyses showing epithelial tumors invading other tissues in patterns comparable to mammalian carcinomas [40]. In many cases malignancies were followed by spontaneous remission, as tumor cells reorganized into normal tissue (or sometimes abnormal but not cancerous structures) [40,56]. Even more striking, in cases where virus-induced tumors (e.g., Lucké carcinomas) from anuran amphibians (*Rana pipiens*) were implanted into actively regenerating newt limbs, tumor regression and subsequent differentiation into regenerating tissue was observed [21,57]. This observation supports regenerative fields possessing important controls over abnormal cells, not only restricting their behavior but also repairing aberrations. Some evidence also suggests this growth control is not restricted to the blastema but also extends to the non-regenerative tissues where metastases were observed [40].

A number of questions still need to be answered regarding this low propensity for tumors in regenerating urodele limbs. One important aspect that remains unclear is whether the tumorigenic differences of regenerating limbs and lens are associated with intrinsic factors not found in other vertebrate cell types. In both lens and limb, tumor avoidance during regeneration could potentially be the result of distinct signals triggered by the injury process itself. It will also be essential to determine whether cells that dedifferentiate actually reverse into completely undifferentiated states. If they in fact only partially lose their differentiated state (and are not completely comparable to undifferentiated cells), the resulting differences in carcinogenesis between urodele and planarians (see below) could be explained by tumorigenic compounds acting upon two distinct "partially undifferentiated" cell populations. Of equal interest is the fact that dedifferentiated cells proliferate extensively in the presence of these carcinogens, suggesting their DNA-damaging effects are not long-lasting. This could imply tremendous potential for DNA repair in these animals, or mechanisms that efficiently coordinate variation in chromosome number as recently documented in adult brain neurogenesis of teleost fish [58]. Unfortunately, there are currently no urodele genomes sequenced, greatly impairing our ability to systematically analyze differentiation profiles or the evolutionary conservation of DNA-repair and tumor suppressor genes.

Just as significant are the similarities between cancer-originating cells and dedifferentiated limb cells. Many similar features (e.g., certain surface molecules) might potentially be conserved between these two cell types, such that both respond similarly to regenerative cues. Most of the current urodele studies on regeneration after chemical insult were performed several years ago, before modern molecular and biological techniques. However, a better understanding of these events could be obtained if recently developed tools such as microarrays, transgenesis, morpholinos, and *in situ* hybridization were applied to the problem. Currently, it is not possible to molecularly alter signaling pathways in urodeles to induce cancer-like growth, confounding our technical ability to unravel molecular origins. Therefore, it is fundamental that existing carcinogenic chemical treatments be standardized, introducing an additional level of control missing from historical studies and probably responsible for discrepancies in outcomes between different studies using the same carcinogen [14,17]. Furthermore, it will be important to characterize where the effects of these chemicals are localized, for example perhaps acting outside the nucleus at the plasma membrane level as has been previously suggested [27]. However, the urodele amphibians still represent a powerful system in which to investigate correlations between cancer and regeneration, and in the future will continue to contribute significantly to our understanding in these areas.

4. Planarian regeneration and cancer

Planarians are free-living organisms of the phylum platyhelminthes (flatworms) well known for their capacity to regenerate entire animals from very small fragments [33–35]. Additionally, this invertebrate can increase its size by adding new cells or reduce it by removing cells, all depending on food availability [59–64]. Such robust adult developmental plasticity is regulated by an undifferentiated cell population scattered throughout the body known as neoblasts. The neoblast is considered an adult somatic stem cell, and it is the only known planarian cell type with the capacity to divide—its progeny produce all known cell types (~40) found in these organisms [30,33–35,63,65]. Neoblasts constantly undergo division and in intact planarians provide new cells to support physiological cell turnover; upon amputation, however, neoblasts extensively proliferate, and their progeny form the regeneration blastema from which missing tissues are regenerated [9,30,33–35,63,65,66]. Thus, the central process of epimorphic regeneration in planarians is associated with activation of resident adult stem cells and not with dedifferentiation as is the case for urodeles [34].

Our current knowledge about cancer and abnormal outgrowth is largely derived from vertebrate studies (particularly mammals), but similar growth aberrations have been reported in almost all major invertebrate phyla (for review see [67]). Despite this commonality, our understanding of the evolution of the regulatory pathways and tumor suppressor genes involved is currently very restricted [68]. The planarian model system, with a recently sequenced genome (*Schmidtea mediterranea*) and well-developed molecular and genetic tools, has great potential to significantly contribute in these areas [30,33–35,63,66,68–70]. Unlike other invertebrate models, planarians have historically been extensively used to test diverse pharmacological and carcinogenic compounds in both whole (non-regenerating) and regenerating adult animals [20,29,36–38,41,42,71]. Results from several studies concluded neoblasts bear a functional resemblance to both adult mammalian stem cells and early-stage embryonic cells; particularly, neoblasts respond to carcinogenic agents by forming benign and malignant tumors, as well as teratomas [20,36–38,41].

Freshwater planarians also display the major phenomenologies of mammalian chemically induced carcinogenesis (those involving the initiation and promotion of neoplastic disease progression), which led previous researchers to propose planarians as a good system for *in vivo* teratogenesis research [20,29,36,37,41]. Like chemically induced tumorigenesis in urodeles, the effects of these compounds in planarians are influenced by many variables (e.g.,

exposure time, frequency, method of administration, toxicity, etc.), making interpretation of the results occasionally difficult. Despite chemical-dependent variability, certain treatments consistently lead to growth abnormalities (such as inhibition of regeneration, abnormal regeneration and regeneration of amorphous structures, or aggressive lethal tumors in non-regenerating animals). Thus, planarian carcinogenesis resembles urodele carcinogenesis, but with at least two important distinctions: (i) most planarian treatments involved soaking entire worms or regenerating fragments rather than local application of compounds as seen in urodeles, and (ii) planarian tumor resistance was not reported as in the amphibian literature. A fascinating exception has been reported in *Dendrocoelum lacteum*, a planarian species with limited regenerative capacities, where a differential response to chemically induced carcinogenesis exists between regenerative (anterior) and non-regenerative (posterior) tissues (Fig. 2B); additionally, anterior regeneration is capable of turning posterior infiltrating tumors into differentiated accessory organs such as the pharynx, which suggests the presence of regulatory long-range signals [16]. However, most planarian studies seem to directly link chemical carcinogens to stem cell response, as neoblasts are the only adult proliferating cells in these organisms.

Thus, planarians offer a simplified model for studying chemically induced tumorigenesis, both within the complexity of the entire organism and during the regeneration of multiple tissues. As a whole, the data demonstrate the ability of planarians to form abnormal growths (as compared to tumor resistance in urodeles), underscoring the differential responses to carcinogenic substances among regenerative tissue types. Furthermore, when combined with new methods to molecularly control cancerous growth in planarians (see below), the extensive body of historical data and seemly unlimited regenerative ability make the planarian model system ideal for systematically investigating connections between cancer and regeneration. By taking advantage of this unique combination, modern examination of Waddington's theory in planaria could significantly advance our understanding of how regenerative tissue is able to strictly regulate growth and control malignancies.

5. The molecular basis for linking regeneration and cancer

Comparisons of gene expression patterns between cancer and wound repair have revealed important differences in several pathways, from those associated with hypoxia-inducible factor and insulin-like growth factor-I, to genes regulating morphogenesis (e.g., *CRYM*, *TCF21*, *CTGF*, etc.) and glycolysis (e.g., *PGK1* and *HK1*) [19]. For instance, a list of candidate genes (e.g., *notch*, *slug*, *mitf*, *EDNRB*, etc.) associated with melanocyte development, regeneration and cancer has been compiled [18]; but all these genes remain to be characterized in large-scale regenerative models. In urodeles, modern molecular techniques need to be applied to release the full potential of these model systems for studies associated with carcinogenesis and regeneration. However, many candidate genes and signaling pathways associated with cancer have been identified in planarians, and the molecular and genomic tools are currently being applied [68,72]. Therefore planarians are potentially the first large-scale regenerative model for dissecting the molecular details of both regeneration and cancer in adults.

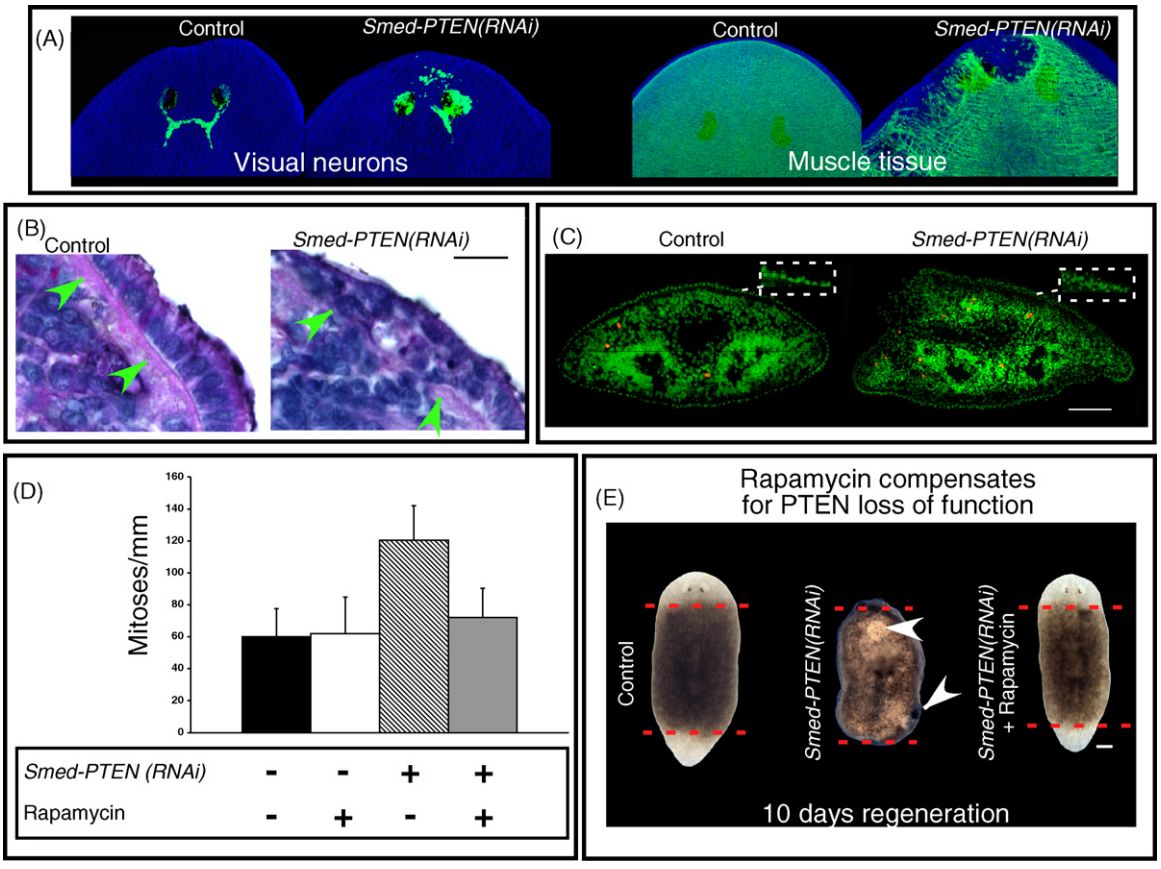

Fig. 3. Loss of planarian PTEN leads to growth aberrations. *S. mediterranea* PTEN loss-of-function by RNAi. (A–C) *Smed-PTEN* RNAi effects: (A) loss of tissue maintenance, (B) basement membrane disruption (green arrows) and the presence of invasive cells (sections stained with hematoxilin and eosin), and (C) an increase in proliferative activity (sections stained with the nuclei marker green sytox) and mitotic cells (red dots). Insets show the transformation of the epithelial monolayer into a multilayer. (D and E) Rapamycin treatment compensates for *Smed-PTEN* loss of function, (D) preventing abnormal proliferation while keeping basal tissue-maintenance levels and (E) restoring regeneration capabilities. White arrows highlight growth aberrations. Data from [72].

Recently, we discovered that using RNA interference (RNAi) to downregulate an evolutionarily conserved tumor suppressor gene in *S. mediterranea*, *Smed-PTEN*, leads to abnormal neoblast proliferation and lethal phenotypes (Fig. 3) [72]. An important regulator of the PI3K signaling pathway, PTEN is among the most commonly mutated genes in human cancer. In planarians, loss of PTEN function is characterized by tissue disorganization, disruption of basement membrane integrity (alteration of epithelial–mesenchymal interactions), and the presence of abnormal cells that invade distant tissues to form aggressive and eventually lethal ectopic outgrowths [72]. This phenotype is consistent with chemically induced neoblastomas in planarians [36]. In addition, neoblasts showed an impaired capacity to differentiate after *Smed-PTEN* RNAi [72], likely related to the abnormally invasive cells contributing to the epithelial dysplasias associated with advanced RNAi phenotypes (as well as the carcinogen-induced neoplasms) [37]. Just as with carcinogenic treatments, regeneration could be inhibited in animals subjected to *Smed-PTEN* RNAi, indicating that under certain conditions PTEN is also required for regeneration. A remarkable finding from these analyses was the striking regulatory conservation of the tumor suppressor *function* of planarian PTEN, which is similar to its mammalian counterpart but is absent in other invertebrates [68]. Thus, the data suggest that molecular disruption of the PI3K signaling pathway in planarians results in abnormal stem cell proliferation, alteration of epithelial–mesenchymal interactions, and cellular infiltration into different tissues that altogether have been associated with vertebrate cancer development [73]. This also fits well with the previous hypothesis relating cancer to an incomplete regenerative process [1,10,13,15,16,19].

A highly desirable, yet still theoretical, cancer therapy centers on identifying and destroying abnormal cells without disturbing homeostasis. Indeed, leukemia-initiating cells can be specifically abrogated in PTEN loss-of-function mice following rapamycin inhibition of the multicomplex protein TOR (a downstream component of the PI3K pathway) [74]. Components of the PI3K pathway are conserved in planarians, and rapamycin exposure following *Smed-PTEN* RNAi was similarly able to prevent phenotypic effects by specifically targeting abnormal proliferative neoblasts while maintaining the basal mitotic activity required for cell turnover [72]. Rapamycin treatment not only preserved homeostasis but also reestablished regeneration in PTEN loss-of-function animals. Currently, our lab is intensively pursuing additional characterizations of the PI3K pathway, as well as different RNAi strategies exploring the genetic–molecular connections between cancerous growth and regeneration.

6. Concluding remarks

Controlling cell growth is clearly a central task in both cancer and epimorphic regeneration. Strangely, the regulatory mechanisms and strategies that animals have evolved to deal with cell growth regulation appear to have the capacity to either destroy or reconstruct. For instance, in mammals chronic epithelial injury often precedes malignant transformation, while in urodeles and planarians persistent damage generally ends merely with functional repair. Injury response always entails attempts to repair the damage, but the fundamental differences probably lay in the coordination of such responses rather than in structural or species-specific capabilities. In planarians, both carcinogenic chemical insults and manipulation of conserved cancer-associated signaling pathways have been shown to affect stem cell population behavior, leading to abnormal proliferation or even propagation failures. Conversely, in urodeles tumorigenesis rarely follows carcinogen exposure, although the reasons for this (non)response are unknown. It can be argued that urodele tumor resistance is a byproduct of their intrinsic developmental plasticity, which is what allows cells to reverse differentiated states and gain progenitor-like status. A central theory in the field remaining to be investigated is whether the regeneration blastema in planarians displays a similar cancer resistance. If true, this would be a strong endorsement for the validity of Waddington's theory that regenerative fields underlie the mechanisms driving uncontrolled growth. In the near future, animal models with extensive regenerative capacities need to be studied with an eye towards cancer mechanisms, using the rapidly increasing number of molecular and genomic resources that have enhanced our ability to manipulate cell growth and dissect the machinery involved. Possibilities for eventual clinical applications make the prospects of exploring the role regenerative processes play in creating and (more importantly) inhibiting malignancies ever more exciting.

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