Review

Therapeutic resistance and cancer recurrence mechanisms: Unfolding the story of tumour coming back

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Cancer recurrence is believed to be one of the major reasons for the failure of cancer treatment strategies. This biological phenomenon could arise from the incomplete eradication of tumour cells after chemo- and radiotherapy. Recent developments in the design of models reflecting cancer recurrence and *in vivo* imaging techniques have led researchers to gain a deeper and more detailed insight into the mechanisms underlying tumour relapse. Here, we provide an overview of three important drivers of recurrence including cancer stem cells (CSCs), neosis, and phoenix rising. The survival of cancer stem cells is well recognized as one of the primary causes of therapeutic resistance in malignant cells. CSCs have a relatively latent metabolism and show resistance to therapeutic agents through a variety of routes. Neosis has proven to be as an important mechanism behind tumour self-proliferation after treatment which gives rise to the expansion of tumour cells in the injured site via production of Raju cells. Phoenix rising is a pro-recurrence pathway through which apoptotic cancer cells send strong signals to the neighbouring diseased cells leading to their multiplication. The mechanisms involved in therapeutic resistance and tumour recurrence have not yet been fully understood and mostly remain unexplained. Without doubt, an improved understanding of the cellular machinery contributing to recurrence will pave the way for the development of novel, sophisticated and effective anti-tumour therapeutic strategies which can eradicate tumour without the threat of relapse.

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1. Introduction

Cancer is not a modern disease, and a huge body of evidence attests to the existence of cancer throughout recorded history. The origin of the word cancer dates back to ancient Greece and the writings of Hippocrates who first applied the term carcinos, meaning crab, to describe solid malignant tumours. The rationale for using the crab analogy was the appearance of extended projections of a tumour that are similar to a crab (Pavlidis and Karpozilos 2011; Manohar 2015). Since the discovery of cancer, extensive efforts have been devoted to cure the disease. Nevertheless, the field of oncology has not seen any definitive treatment. One of the prime reasons for the failure of therapeutic scenarios is deficient knowledge on the mechanisms underlying drug resistance of tumour cells. With ongoing breakthroughs in clinical oncology, several targeted therapies are now available. But, neoplastic cells harness the power of a variety of resistance mechanisms to rebuild themselves after initial treatment (Tsuruo *et al.* 2003; Felsher 2006). Cancer recurrence occurs when a portion of primary tumour cells escape the devastating effects of drugs, and remain in small areas which could not be detected even by clinical diagnostic tests. With the passage of time, these surviving cells undergo numerous genetic alterations

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and ultimately generate new tumour cells. The time of cancer relapse can vary ranging from weeks and months to even years after diagnosis of the initial tumour. It is likely that a minority of cancer cells still remain in place after surgery. Similarly, some tumour cells can survive following chemotherapy and radiotherapy, become resistant to therapy, and in the long run form new tumours (Baker *et al.* 2005). The rate of cancer recurrence depends on various factors including age, sex, cancer type, etc. The lengthy process of cancer treatment and the probability of recurrence can cause a lot of problems for the final recovery of patients. As a result, there is a clear need to develop a deeper understanding of the mechanisms implicated in recurrence. Further insight into the mechanistic details of tumour relapse paves the way for the development of more effective therapeutic approaches while minimizing adverse effects.

In this article, we provide a brief overview of three mechanisms which contribute to the acquisition of resistance by cancer cells against current therapeutic regimens and result in the recurrence of malignancy through various routes. The current body of knowledge on these resistance-generating mechanisms opens up new avenues for advancing our understanding of the causes and conditions leading to tumour reconstruction. From the practical viewpoint, this information will help researchers plan more powerful strategies for the treatment of a variety of tumour types. We will describe how cancer stem cells are formed and by what means they induce tumour resistance against therapeutics. Thereafter, we take a look at neosis through which cancer cells apply the characteristics of cellular senescence to escape treatment. In the last section, we will see although apoptosis has long been well recognized as a suppressor of cancer progression, it can also play crucial roles in cancer recurrence through the phoenix rising pathway.

2. Cancer stem cells

In 1937, Furth et al. for the first time measured the abundance of malignant cells which could contribute to the maintenance and survival of blood tumours. They demonstrated that all cells of a malignant neoplasm are not able to form tumours, but this capacity is limited to a subset of cells called cancer stem cells (CSCs) (Furth *et al.* 1937). During 1960s and 1970s, the concept of cancer stem cell was further supported by new evidence indicating that the heterogeneity of tumour cells might be a player in tumour re-formation *in vivo* (Killmann *et al.* 1963; Clarkson *et al.* 1970; Gavosto 1970; Clarkson and Fried 1971). The CSC hypothesis posits that a small population of cancer cells called CSCs or TICs (tumour-initiating cells) are responsible for the early formation, progression, recurrence, and drug resistance of tumours (Welte *et al.* 2010; Rahman *et al.* 2011; Rasheed *et al.* 2011; O'Connor *et al.* 2014). CSCs are immortal and pluripotent cells that can drive the generation of new tumour cells (Chen *et al.* 2013). A large body of convincing evidence indicates that CSCs arise from normal stem cells, progenitor cells, or differentiated cells through accumulation of genetic mutations and subsequent genomic instability (Han *et al.* 2013). All CSCs share two general characteristics which include:

Self-proliferation: CSCs, like normal stem cells, are capable of self-proliferation. But they show a significant difference from normal stem cells in that they have an uncontrollable self-proliferative activity. An individual CSC divides asymmetrically and produces one CSC and one differentiated tumour cell. The latter cells form the major part of the tumour (Jordan *et al.* 2006). To date, CSCs have been detected in a wide variety of cancers including malignancies of the blood, breast, brain, ovary, kidney, to name a few (Bussolati *et al.* 2008a; Shen *et al.* 2008a, 2008b; Alvero *et al.* 2009; Bussolati *et al.* 2009; Ricci-Vitiani *et al.* 2010; Zhao *et al.* 2010).

Slow proliferation or inactive state: Inactivation is not a general property for cells. However, some tumour cells adopt three different forms of slow proliferation, active state, and inactive state. For example, inactive leukaemic stem cells have been observed in a chronic myeloid leukaemia (CML) mouse model. Furthermore, clonal tracing technique which allows researchers to track the progeny of the individually marked tumour cells *in vivo* has revealed that a large proportion of CSCs in acute myeloid leukaemia (AML) and colon cancer remain in inactive state for a long period of time causing their less efficient response to therapy. Hence, the reactivation of inactive CSCs may promote tumour recurrence even decades after complete treatment of the disease (Allan *et al.* 2007; Ojha *et al.* 2015).

Considering that there is a close relationship between CSC, tumorigenesis, metastasis, drug resistance and cancer recurrence, chemotherapy through targeting CSCs represents a valuable and useful approach both for the complete eradication of malignant cells and for preventing their spread to other parts of the body (figure 1). The isolation of CSCs is a prerequisite for targeting them. The isolation of CSCs from bulk tumour cells is performed through the use of different approaches. Currently, fluorescence-activated cell sorting (FACS) is the most commonly used methodology for the identification of CSCs (Khan *et al.* 2015). This method functions based on the identification of specific cell surface or intracellular markers. Thus far, a variety of markers have been found which can distinguish CSCs from other tumour cells as well as normal stem cells. CSCs are mostly characterized by the expression of cell surface markers such as CD133, CD44, and intracellular markers such as ALDH1 (aldehyde dehydrogenase 1 enzyme) (Singh *et al.* 2004; Huang *et al.* 2009). However,

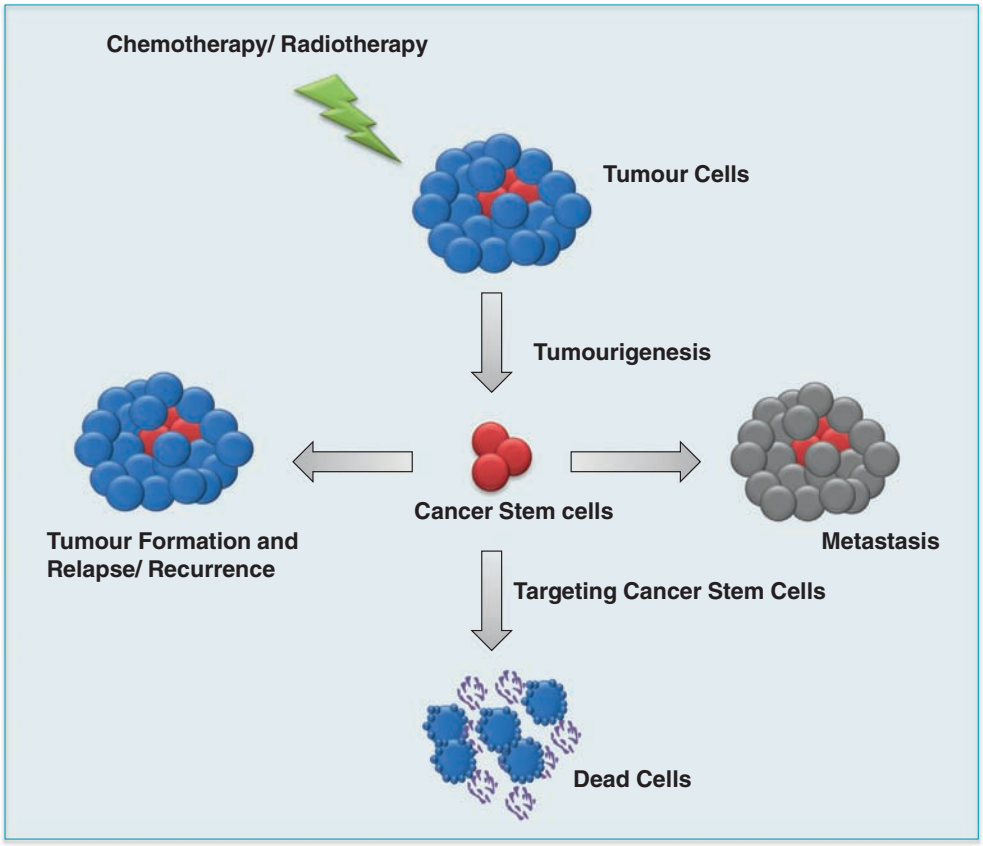

Figure 1. Optimized chemotherapy by targeting CSCs. Tumours are heterogeneous with populations of CSCs distributed in different parts within the tumour. Chemo- and radiotherapy kill tumour cells, but CSCs show therapeutic resistance and continue to live even after being exposed to chemo- and radiotherapeutic agents. This therapeutic resistance, on one hand, leads to the enhanced metastatic invasion of tumour cells into other tissues of the body and, on the other hand, raises the chance of recurrence and tumour-re-formation. But, when chemo- and radiotherapeutic intervention targets CSCs, tumour relapse is much likely to be avoided.

the expression of CSC-specific markers highly depends on the type of cancer. It is worth mentioning that all CSC-specific markers have not been fully identified and all CSC types do not express all of these markers. Even cell types other than CSCs are also capable of producing some of these markers. Accordingly, although these markers can serve to detect cell populations enriched by CSCs, they do not have the capacity to definitely discriminate between CSCs and other cell types (Medema 2013). Because of therapeutic resistance, sorting the side population (SP) of CSCs in flow cytometry can also be achieved via exclusion of intracellular Hoechst 33342 dye. However, this method is able to identify a minor fraction of CSCs that are not sufficient for further experimentation. Furthermore, SP cells are protected from Hoechst cytotoxic effects by their unique membrane transport features, while non-SP cells cannot be protected and consequently do not grow. As a result, discriminating between SP and non-SP cells may be attributed mainly to an artifact of dye cytotoxicity rather than inherent stem cell characteristics. The limited capacity of CSC selection through isolation of drug-resistant cells has prompted researchers to find approaches that could identify a larger number of CSCs (Shen *et al.* 2008a, 2008b; Yanamoto *et al.* 2011). Sphere-forming assay is a widely-used in vitro methodology for the isolation and characterization of CSCs. This approach identifies self-renewing cell lineages that are able to grow in suspension (Dontu, Abdallah *et al.* 2003). Several pieces of evidence have suggested that the sphere culture system is highly efficient in the isolation of CSCs from a variety of cancer cells and solid tumours. This method has been indicated to successfully separate CSCs from non-CSCs when there is no prior knowledge of CSC-specific markers, as is the case for most CSCs. The exploitation of a serum-free culture medium supplemented with optimal amounts of mitogens such as basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF)

can lead CSCs to be enriched in spheres (Hueng *et al.* 2011). The spheroid body culture has shown success in the isolation and enrichment of potential CSC subpopulations from a variety of primary tumours. The spheroid body-forming cells from different malignancies including ovarian, breast, gastric, liver, and brain cancers have exhibited stem-like features with the expression of specific CSC markers (Zhong *et al.* 2010; Hueng *et al.* 2011; Liu *et al.* 2013). The concept of brain CSC has emerged partly from studies indicating that brain tumours harbor sphere-forming cells which express neuronal and glial markers when cultured in serum-free mitogen-containing culture medium (Chen *et al.* 2010). Later reports provided strong support for the notion that such cells could be expanded in stem cell culture media and reconstitute tumours *in vivo* (Galli *et al.* 2004). Despite its promise for CSC isolation, sphere formation assay suffers from some drawbacks. The spheres formed by tumour cell proliferation are difficult to be distinguished from high-density cell aggregates (Pastrana *et al.* 2011). Also, this approach can detect only proliferating but not quiescent CSCs. Actually, sphere formation assay is a proliferation-based methodology that mostly allows the expansion of actively dividing CSCs. However, the rapid *in vitro* expansion of quiescent CSCs in the presence of growth factors is severely hampered by their slower cell cycle kinetics. Furthermore, the self-renewing potential of non-stem cancer cells is an issue that bears further consideration. Although self-renewal is recognized as a unique characteristic for CSCs, a variety of tumour cell lines have been reported to exhibit sphere formation capacity. This observation suggests that not all tumorspheres derive from CSCs (Kondo *et al.* 2004). The limitations of sphere-forming assay have led researchers to introduce methodologies that can surmount some of the current obstacles. In line with this, some novel approaches have been developed that include bioengineering strategies using patterned substrates to mimic the *in vivo* extracellular matrix, adherent two- and three-dimensional culture systems, and co-culture configurations with various niche components (Vunjak-Novakovic and Scadden 2011). In spite of the development of these alternative strategies, sphere formation assay is still of particular importance for CSC isolation and its subsequent characterization; thus, the further examination of sphere formation plays an important role in CSC research.

CSCs apply different mechanisms to develop resistance to treatment. One of the major events playing role in CSC resistance to chemotherapy is releasing anti-cancer drugs out of the cell through plasma membrane ATP-dependent pumps such as breast cancer resistance protein (BCRP/ABCG2), multidrug resistance-associated protein 1 (MRP1/ACC1), and P-glycoprotein 1 (MDR1/ABCB1) (Alisi *et al.* 2013). Also, the enhanced levels of ALDH1 contribute to CSC drug resistance. This cytosolic enzyme catalyzes the oxidation of aldehyde compounds and triggers resistance to cyclophosphamide (Abdullah and Chow 2013). Many chemotherapeutic drugs kill tumour cells via inducing apoptosis. Some CSCs become drug resistant by producing anti-apoptotic molecules such as Bcl-2 (Abdullah and Chow 2013). On the other hand, the niche of CSCs and their surrounding microenvironment which provide essential substances and factors for long-term survival and self-proliferation can protect them from drug-induced apoptosis. For instance, microenvironment is a critical determinant for the non-treatability of most B cell-related malignancies (Allan *et al.* 2007; Chen *et al.* 2013). CSCs can acquire resistance to genotoxic therapies such as ionizing radiation through different routes including increased activity of the DNA damage checkpoints and enhancement of the DNA repair capacity (Medema 2013). Recent studies have shown that CSCs positive for CD133 – a cell surface glycoprotein and a molecular marker for brain tumour-initiating cells – derived from human glioblastoma (GBM) mediate a higher resistance to radiation compared with CD133-negative cells (Sakariassen *et al.* 2007). This increased resistance of CD133-expressing cells is attributed in part to improved DNA damage response and accelerated repair of the damaged DNA. The number of $CD133^+$ CSCs has been demonstrated to increase in established glioma cell lines and xenograft tumour-bearing mice following exposure to high-dose radiotherapy leading to the formation of more invasive tumours. It is not clear why radiation stimulates the division of $CD133^+$ cells (Bao *et al.* 2006; Sakariassen *et al.* 2007). Moreover, the activation of mesenchymal stem cell (MSC) transcription factors induces epithelial-mesenchymal transition (EMT) in CSCs through a variety of signaling routes such as the Hedgehog (Hh) pathway. The induction of EMT by these transcription factors is followed by drug resistance, enhanced invasiveness, improved growth of tumour cells and finally relapse of the disease (Singh and Settleman 2010; Wang *et al.* 2015).

Recent investigations have highlighted the importance of autophagy as a critical factor for the survival and drug resistance of CSCs (Ojha *et al.* 2015). In addition, autophagy fulfills a key role in establishing a dynamic balance between CSCs and normal stem cells. Autophagy is a conserved catabolic process which contributes to cell survival under stress conditions such as starvation, hypoxia, and exposure to chemo- or radiotherapy (Sui *et al.* 2013). Once activated, autophagy results in the formation of autophagosomes which enclose damaged organelles and cellular components. Later, autophagosomes merge with lysosomes to generate autophagolysosomes. Lytic enzymes degrade contents of autophagolysosomes in order to release essential nutrients for cellular metabolism such as amino acids and fatty acids (Kroemer 2015). Autophagy plays a dual role in the process of tumorigenesis. On one hand, it acts as a tumour suppressive mechanism by preventing the accumulation of damaged organelles and proteins. On the other hand, it makes contribution to the survival of tumour cells under starvation and hypoxia via maintaining homeostasis. Cancer cells activate the

autophagy pathway in response to cellular stress or increased metabolic demand. Autophagy-mediated raise of stress tolerance is known to retain the process of energy production, and thereby promotes the growth and drug resistance of tumour cells. Pre-clinical studies have revealed that autophagy inhibition results in an enhanced susceptibility to chemotherapeutic compounds and elevated rate of cell death in various cancers (Gewirtz 2014; Ojha *et al.* 2015). It is evident that obtaining further insight into the factors involved in the pathogenesis of CSCs paves the way for the successful design of novel and efficient therapeutic strategies.

3. Neosis

Sundaram *et al.* exposed a mouse embryonic fibroblast (MEF) cell line C3H10T1/2 to genotoxic agents such as etoposide and radiation and observed a mode of cell division with atypical karyokinesis; a phenomenon which was called neosis. Later, modern video equipment and computer analysis were used to study this cellular event in greater detail (Sundaram *et al.* 2004). To date, neosis has been found and investigated under different conditions in various cells. Neosis is a type of cell division that could underlie the resistance of tumour cells to chemo- and radiotherapy (Navolanic *et al.* 2004). Cell division is conventionally placed in two categories of mitosis and meiosis. During mitosis, a maternal diploid cell is divided into two identical daughter cells. The reiterative mitotic divisions of zygote are responsible for the growth, development, and survival of multicellular organisms. By contrast, meiosis is known as a reductional division which produces haploid cells with a new combination of alleles giving rise to gametes. In both types of cell division, the nuclear envelope is first disassembled and then assembled when chromosome pairs are equally segregated into new daughter cells (Rajaraman *et al.* 2007).

Normal somatic cells have a limited longevity called mitotic life span (MLS). At the end of their MLS (after approximately 60-70 mitotic divisions), somatic cells acquire a series of accumulated genetic mutations which is followed by increased genomic instability. These alterations lead to cellular senescence and the cells lose their capacity to proliferate. This phase is characterized by the formation of multinucleate and polyploid giant cells (MN/PG) cells and unresponsiveness to growth- stimulating signals (Roninson *et al.* 2001; Dimri 2005). Telomere shortening and the problem of replication of the linear chromosome ends are considered as key factors implicated in driving cells to senescence and losing the ability to carry out mitosis. Also, when normal and cancer cells are exposed to DNA-damaging substances, accumulating mutations and subsequent enhanced genetic instability propel the cells toward senescence followed by the generation of MN/PG cells. MN/PG cells have a high genomic instability and are not capable of responding to growth signals. They arise from stresses occurring in different steps of senescence, have a limited MLS, and die after a defined number of mitotic divisions. Generally, MN/PG cells die through apoptosis (programmed cell death) or because of deficient apoptosis are destroyed via mitotic crisis or catastrophe (Sundaram *et al.* 2004; Rajaraman *et al.* 2005; Rajaraman *et al.* 2006). Mitotic catastrophe is recognized as a tumour suppressive mechanism during which the nuclear envelope is disrupted and mitotic spindle checkpoints are activated. Then, cells with damaged DNA go into the mitotic phase, but cannot successfully complete mitosis to the end which finally results in cell death. However, some cells may escape mitotic crisis and produce a certain type of mononuclear cells with a longer MLS. This process is called neosis and the generated mononuclear cells are referred to as Raju cells. The onset of neosis requires the presence of senescent MN/PGs which are known as neosis mother cells (NMCs) (Sundaram *et al.* 2004; Rajaraman *et al.* 2005; Rajaraman *et al.* 2006).

Neosis is a reductional division in which nuclear budding is followed by nuclear division without changes in the structure of the nuclear membrane. Afterwards, asymmetric cytokinesis results in the appearance of aneuploid and mitotically active Raju cells which in turn mature into tumour (transformed) cells (Sundaram *et al.* 2004; Rajaraman *et al.* 2005; Rajaraman *et al.* 2006). During neosis, NMCs prevent complete disintegration of the nuclear envelope in karyokinesis. This blocks the activation of spindle checkpoints and occurrence of mitotic crisis. In fact, karyokinesis via nuclear budding and asymmetric cytokinesis are considered as a transitional status for Raju cells to achieve genomic stability. Most likely, telomerase reactivation is the leading cause of this relative stability. Telomerase expression extends the MLS of Raju cells. Then, cells continue symmetric mitotic divisions and generate a cell clone in which daughter cells mature by size increase and a series of phenotypic and genotypic changes turning to tumour cells. In general, neosis is a fundamental intermediate process for the generation of aneuploid and polyploid tumour cells (Rajaraman *et al.* 2005).

Each NMC produces over ten Raju cells. These cells are different from NMCs in phenotypic and genotypic features and might be quite different from each other. Individual neotic clones have various characteristics suggesting that neosis triggers heterogeneity in tumour cells. As a result, different Raju cells show different levels of resistance and cells with a longer life span are more resistant. Finally, death of NMCs after the appearance of Raju cells terminates the unstable mitotic genome while giant cells have left behind cells appropriate for tumour re-formation (Rajaraman *et al.* 2005).Therefore, damaged and genomically unstable tumour cells not only multiply through conventional mitosis but also increase in number by implementing neosis as a new type of cell division. Accumulating evidence indicates that neosis takes place several times during tumour growth. It gives rise

to Raju cells as tumour precursor cells and is responsible for the drug resistance of cancer cells (Rajaraman *et al.* 2006). MN/PGs are not able to appropriately divide the genomic material into daughter cells via mitosis because they have one centrosome or multiple centrosomes. Therefore, they generate Raju cells through multiple times of the nuclear envelope disassembly and re-assembly. These Raju cells are called tumour-initiating Raju cells (TIRC) and this phase is termed primary neosis (P-neosis). TIRCs are unique because they transiently display stem cell features, inherit genomic instability, and then turn to tumour cells. The following neosis divisions are secondary or tertiary neosis (S/T neosis) and the resultant Raju cells are referred to as Tumour Rejuvenating Raju Cells (TRRCs) (Sundaram *et al.* 2004; Rajaraman *et al.* 2007). Figure 2 illustrates the steps leading to neosis after a cell is exposed to genotoxins including chemo- and radiotherapeutic agents and depicts how TIRCs contribute to tumour progression.

In actual, during the early neosis cells choose this type of cell division to stay alive rather than moving toward mitotic crisis. In these cells, the senescence-associated cell cycle checkpoints are defective, a number of tumour suppressor genes have been inactivated, and some oncogenes have been activated. Accordingly, they can grow again and escape the mitotic crisis through entering secondary or tertiary neosis. Therefore, the neosis principle supports the notion that tumour cells are not immortal. Some may die due to mitotic crisis or mutations, while some other cells can divide multiple times by undergoing neosis (Rajaraman *et al.* 2006). It is still unclear how mitotically active mononuclear Raju cells evolve from non-viable polyploid cells. However, it is believed this event could be triggered by factors such as the accumulation of genetic and epigenetic changes, defective function of checkpoints associated with senescence, and malfunction of tumour suppressor genes such as p53/pRB/p53Ink4a (Singh *et al.* 2010).

It has been demonstrated that neosis is also correlated with resistance induced by exposure to chemotherapeutic drugs. A considerable body of evidence underlies the dramatic importance of neosis as one of the mechanisms underlying cancer recurrence. A variety of studies have revealed that the progeny of Raju cells produced by neosis are more resistant to treatment by several orders of magnitude. These cells can withstand chemotherapy- and radiotherapy-induced mitotic damages. The association between neosis and drug resistance becomes more obvious when a number of senescent and multinucleated cells, giant polyploid cells, and small cells with large nuclei have been observed among tumour cells remaining after treatment (Sundaram *et al.* 2004; Rajaraman *et al.* 2005; Rajaraman *et al.* 2007; Zhang *et al.* 2014). Neosis is recognized as a distinct approach not only for explaining the mechanism underlying tumour formation, but also for describing the different steps which trigger therapeutic resistance and cancer recurrence. In both scenarios, remaining cells serve to regenerate cells and re-commence cancer; though here in neosis an atypical division leads tumour to come back.

4. Phoenix rising

Phoenix rising is a mechanism through which dying cells send growth- and division-inducing signals, and thereby produce new cells (Li *et al.* 2010; Huang *et al.* 2011; Liu *et al.* 2014). When a tissue is injured, cells present in the damaged site grow and multiply in order to replace the damaged ones. Stem cells present in and around the injury site play crucial roles in wound healing (Gurtner *et al.* 2008). It is believed that factors released from wound cause the migration of stem cells towards the injury site as well as their differentiation and proliferation (Mosser and Edwards 2008). Injury to mammalian tissues has been recognized as an inflammatory agent and many studies introduce immune cells as the first responders to inflammation. In particular, neutrophils and macrophages were viewed as the primary cells implicated in wound healing. In line with this, attention was focused on investigating the role played by immune cells like macrophages as well as their released cytokines and growth factors in the process of wound healing. Later, experiments on neutrophil- and macrophage-lacking mice shed light on the fact that none of these cells are essential for wound healing (Martin *et al.* 2003; Mosser and Edwards 2008). It has recently been realized that factors associated with cell death may effectively contribute to the recovery of injured tissue. Actually, apoptosis-inducing compounds have been demonstrated to trigger a paracrine cascade which ultimately leads to the proliferation of stem cells (Li *et al.* 2010). Apoptosis is a programmed cell death which plays indispensable roles in the embryonic development, tissue homeostasis, as well as in cancer therapy (Liu *et al.* 2014). Although seemingly paradoxical, apoptotic cells could induce tissue repair in a process called compensatory proliferation. This phenomenon has been observed in a wide variety of organisms ranging from lower animals such as Hydra and Drosophila to mouse and human (Huang *et al.* 2011). To examine the effects caused by dying cells on inducing the growth of neighboring living cells, mouse embryonic fibroblasts (MEFs) killed by radiation were used to mimic dead cells present in the wound. The culture of a variety of tumour cell lines (including stem cells and cancer progenitor cells) in the vicinity of killed MEFs obviously resulted in the growth and increase of the number of tumour cells. Caspase 3 and caspase 7 which are important players in the final stages of apoptosis are known to be critical determinants of cell division and apoptosis (Li *et al.* 2010; Huang *et al.* 2011; Liu *et al.* 2014). The activation of these caspase molecules triggers a cascade through cleaving and activating the phospholipase enzyme (iPAL2) giving rise to the elevated levels

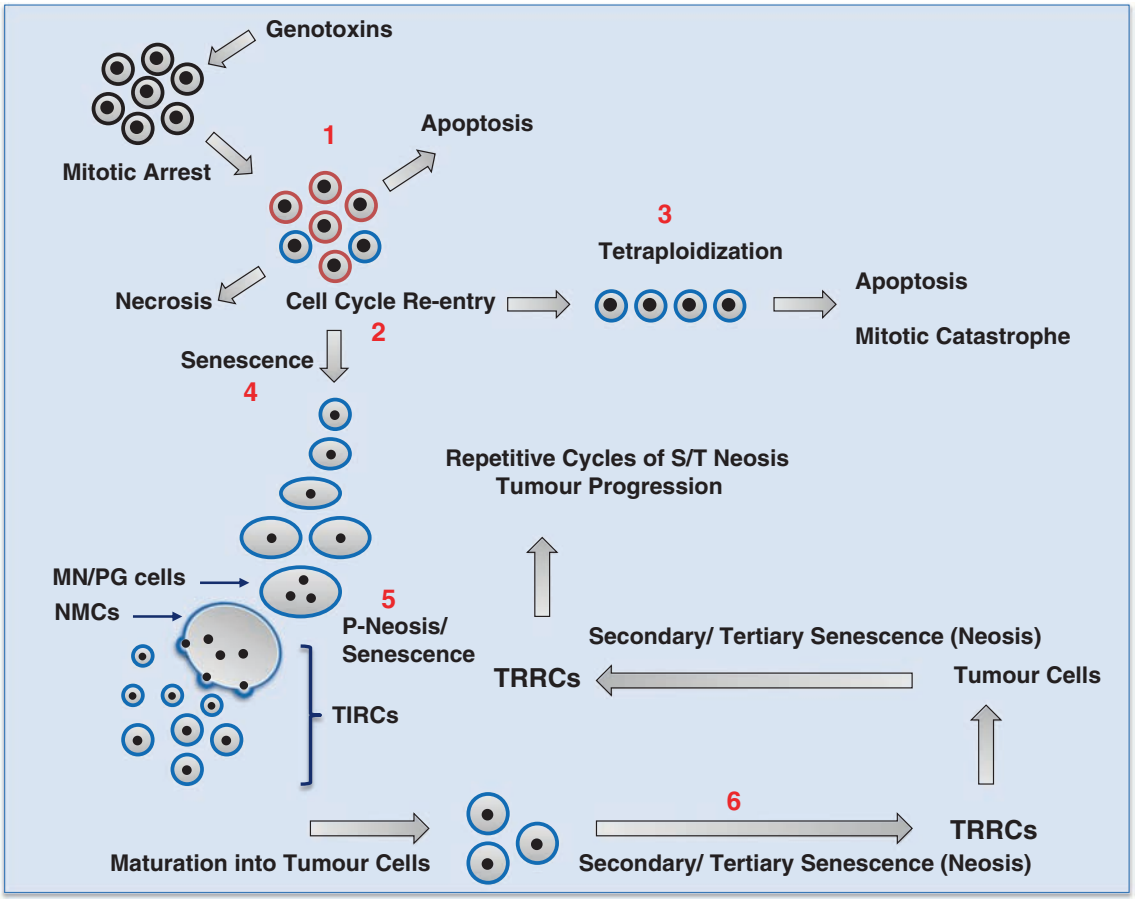

Figure 2. Neosis and its role in tumour growth and recurrence. Cell exposure to genotoxins can determine different fates for the cell including cell cycle re-entry, apoptosis, necrosis, and premature senescence entry. However, a small number of cells can undergo neosis and generate tumour initiating Raju cells (TIRCs), which are known as the precursors of tumour cells. Raju cells can revive the reservoir of genotoxin-resistant tumour cells through production of Tumour Rejuvenating Raju Cells (TRRCs).

of prostaglandin E2 (PGE2). PGE2 could function as a growth inducer and leads to tissue repair by stimulating the growth of local stem cells (Li *et al.* 2010). In Greek mythology, phoenix is a legendary bird that is reborn by rising from its own ashes. This pathway is called phoenix rising since it turns the typically death-causing phenomenon of apoptosis to a life-giving process which contributes to the repair and healing of the injured tissue.

Dying tumour cells have been demonstrated to take advantage of apoptosis to produce growth signals, and rebuild tumours damaged by exposure to radiation. This implies the fact that although phoenix rising is a rapidly functioning and useful route for injury repair, it can also generate resistance to therapy and cause cancer to come back. Actually, cells destroyed by radiation or chemotherapy send messages which trigger the growth and proliferation of few stem and progenitor cancer cells remaining alive in the site. The multiplication of these surviving cells provides the possibility for tumour re-formation and its relapse. Similar to the process described for tissue repair, apoptotic tumour cells activate caspases 3 and 7, activated caspases cleave iPAL2, and the active form of iPLA2 causes the release of arachidonic acid (a polyunsaturated fatty acid present in the phospholipids of the cell membrane) into the cell cytoplasm. The processing of arachidonic acid by different enzymes such as cyclooxygenase (COX) produces various eicosanoid derivatives such as PGE2 which promote the growth and proliferation of cancer stem cells and cancer progenitor cells (figure 3). This can occur through different regulatory pathways of the cell (Bergmann and Steller 2010; Huang, Li *et al.* 2011). The exact mechanism underlying this process is not fully understood, but the Wnt/β-Catenin signaling pathway has been revealed to account for some cases of compensatory proliferation (Bergmann and Steller 2010; Jager and Fearnhead 2012). It is well known that the Wnt/β-Catenin pathway largely contributes to the activation of the genes involved in cellular survival and proliferation (c-myc, cyclin D1,

COX-2), anti-apoptosis (Bcl-2), and angiogenesis (VEGF) and these cellular events can bring about cancer recurrence (Clevers and Nusse 2012).

Recently, different pathways beginning with apoptosis induction and ending with PGE2 release have been investigated and the results of these studies have been applied to present a mathematical model (Liu, Li *et al.* 2014). This model helps in understanding the molecular mechanisms behind the phoenix rising pathway and mimics the effects caused by the expression of a variety of genes - including C3, C7, and NFkB on PGE2 secretion. According to this model, the activation of C3 has a more potent impact than C7 on the secretion of PGE2 and proliferation of remaining tumour cells (Liu *et al.* 2014). In general, phoenix rising like other cancer recurrence strategies adopts the body's natural mechanisms to provide appropriate means for the growth and proliferation of malignant cells. In this context, apoptosis serves for the release of agents capable of fostering cancer cell proliferation. In this manner, dying cells act as inducers for the regeneration of new cells.

5. Conclusion

The return of cancer cells constitutes a huge challenge for cancer therapy. Here, we elaborated on the three different mechanisms associated with cancer recurrence and described their interaction with therapeutic strategies such as chemotherapy. CSCs have been demonstrated to play key roles in tumour relapse. They have a variety of distinguishing characteristics which make them resistant to therapeutics. These attributes include partial latency, the expression of ABC transporters, the ability to interfere with apoptotic signals, DNA repair capacity, autophagy, and triggering survival mechanisms. Tumour cells are not immortal and have a limited MLS. These cells reach senescence and some of them escape death through mutagenesis. Also, emerging evidence lends support to the notion that the self-proliferative capacity of cancer cells is not due to CSC immortality, but results from neosis and the generation of new Raju cells. On the other hand, it has been revealed that dying tumour cells can apply the mechanism of phoenix

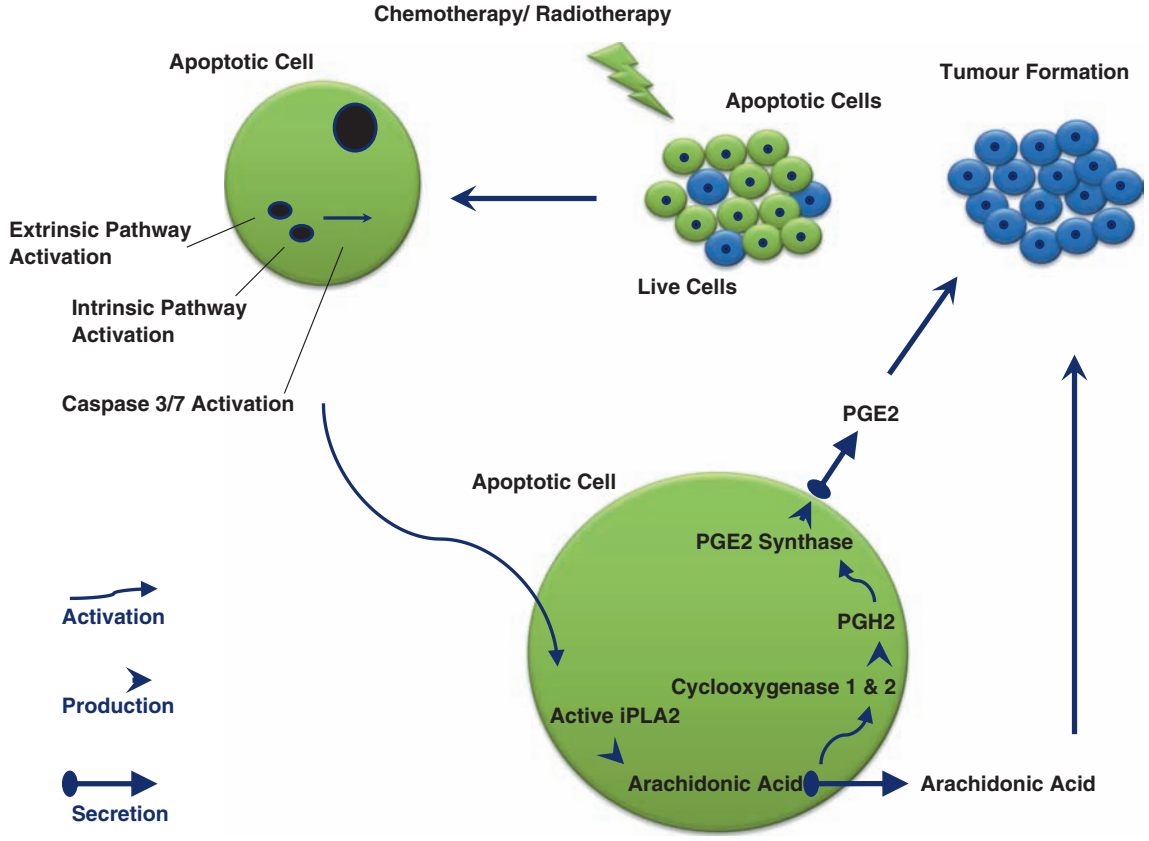

Figure 3. The 'phoenix rising' pathway for cell death-mediated tumour formation. Chemo- and radiotherapy induce apoptosis in some cancer cells as a result of tissue damage. Caspase 3 and caspase 7 become active through intrinsic or extrinsic pathway. These activated caspases give rise to a series of changes in apoptotic cells including iPAL2 activation, arachidonic acid release, and COX-mediated production of eicosanoid derivatives such as PGE2. PGE2 triggers several intracellular signalling pathways leading to the expression of a variety of genes. The functional products of these genes play roles in promoting the re-population of remaining cancer cells and tumour formation.

rising to drive the propagation of remaining cancer cells by secreting/releasing growth-inducers such as iPAL2.

The mechanisms underlying cancer recurrence have not yet been fully uncovered. So, further studies are required to gain a complete picture of recurrence and its underlying mechanisms. Tumour recurrence is of particular interest both theoretically and practically and its understanding faces many problems which remain to be solved. From technical viewpoint, one of the most challenging issues is detection, isolation, and characterization of latent cancer cells in samples derived from patients. These cells could not be identified because they do not possess specific markers. Once isolated, the molecular properties of cancer cells need to be characterized. Currently, this could be achieved by comparative genomic hybridization (CGH) approaches and gene expression array studies. For molecular characterization of tumour cells, their gene expression profile could be compared with CSCs. Another problem of cancer recurrence studies is the lack of an appropriate model. The mechanisms introduced to explain the phenomenon of tumour relapse are largely based on a limited number of experimental models. Accordingly, more models are required to advance our understanding of the basic principles of recurrence. Moreover, the identification of molecular agents involved in the activation of latent tumour cells is a requisite. Also, there is a clear need to more sophisticated imaging techniques for the analysis of tumour recurrence studies *in vivo*. Taken together, current findings on cancer recurrence have opened new perspectives to a more detailed appreciation of cancer progression. Gaining a deeper insight into different aspects of this complex biological phenomenon and its fundamentals will provide a source of hope for the establishment of more effective diagnostic and therapeutic strategies.

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