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Epidermal stem cells and cancer stem cells: Insights into cancer and potential therapeutic strategies

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ABSTRACT

Epithelial keratinocyte regeneration has been exemplified as dependent on a population of cellular progenitors that have retained developmental pluripotency, a latent capacity for proliferation and differentiation with a prolonged lifespan. Recent evidence suggests that the cell populations that regulate the development of normal tissues, and which play vital roles in maintaining the overall homeostasis of the tissue, might be the key target population that is essential for malignant cancer development, thus giving rise to the notion of 'cancer stem cells'. This review examines the leading research into the relationship between adult stem cells in human skin marked by p63αΔN, their putative importance in cancer development, and how we might exploit our evolving knowledge of adult tissue stem cells to aid cancer treatments in the future. Furthermore, the review examines information regarding ataxia telangiectasia mutated (ATM) kinase and key regulatory events that take place on p53, only within putative keratinocyte stem cells that are transcriptionally regulated by p63αΔN.

1. Introduction

The epidermis is a protective barrier subject to an array of genotoxic insults known to be involved in tumourigenesis. Subsequent regenerative cycles depend on a subset of epithelial precursors that resist terminal differentiation and retain their potency for proliferative capacity,[1] namely stem cells. The identification of this subset of stem cells represents an important step towards understanding the events that regulate cellular differentiation of epithelia and the consequences of their subversion. Furthermore, the elucidation of the cell population that is at risk of becoming cancerous is likely to be important in the development of effective therapies. Some of the phenotypes of cancer are similar to the qualities attributed to adult tissue stem cells. The first clues towards the notion of stem cells being the cancerous target were described by cell biologists nearly 30 years ago.[2] Since then, keratinocyte subpopulations have been found to be highly susceptible to the acquisition of oncogenic mutations.[3,4] However, only a small number of skin cancers develop, as most cells acquire mutations that are lost through the normal process of terminal differentiation, which acts a cellular proof-reading mechanism. It has been demonstrated that more than one genetic lesion is required to cause a sustainable tumour. The majority of tumours are clonal in their origin and it has been estimated that 3–5 genetic events in humans and 2–3 in rodents are necessary to transform a normal cell into a cancer cell.[5,6] Thereby, the long-term residents of the epidermis, such as stem cells, are possibly the only cells that have the ability to accumulate the number of genetic hits necessary to result in tumour formation while remaining viable.[7]

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Skin stem cells have been described as either unipotent, implying that they generate a single lineage, or multipotent, meaning that they generate multiple lineages (pluripotent stem cells have not been identified in skin). Skin stem cells usually divide infrequently (slow cycling) to generate either two daughter stem cells that are identical to the founding stem cell (symmetrical division) or one daughter cell identical to the founding stem cell and one with differing capacity (asymmetrical division).[8,9] Elegant studies by Barrandon and colleagues selectively demonstrated that three classes of the epidermal keratinocyte populations comprise the epidermis and that only one class had a true potential to form large colonies, indicated by a high proliferative capacity. The three classes of cells described were holoclones (stem cell-like), meroclones (transit amplifying (TA) cells) and paraclones (terminally differentiated cells), respectively.[10] Treatment with a known carcinogenic stimulus did not culminate in each class liberating cancerous cells. The only class that had the ability to form viable, highly proliferative genetically damaged cells were the holoclones, now known to have stem cell populations.[11] Another example was shown when it was reported that ultraviolet (UV) light induced *TP53* mutations in human interfollicular epidermis.[12] Numerous cells with *TP53* mutations were found in sun-exposed, but clinically normal, human epidermis. Both scattered single cells and clonal patches of mutated *TP53*-positive cells were observed throughout the exposed epidermis.[13–16] The location of large patches of *TP53* mutated cells was found to be selective for stem-cell-rich regions, exemplifying that epidermal stem cells maybe be the only cells that have the capacity substantially to propagate UV-light-induced genetic alterations.[7] The selective survival of epidermal stem cells is not thought to lead directly to cancer, but their progeny have a greater risk of accumulating the further genetic modifications within key tumour suppressor genes that are required to induce tumour formation.

Further discoveries in cancer biology demonstrate that lesions arise from stem cells by selective mutagenic events creating the formation of cancer stem cells that then go on to constitute the formation of a tumour. In these models, cancer stems cells do not represent a majority of the cells within a tumour, but are nevertheless critical for its propagation. Evidence for this is that tumours have also been reported to undergo a differentiation process, giving rise to both TA cells and terminally differentiating cells, which are genetically altered. Many reports of cancers possessing differentiated cell types have long been documented in a wide compendium of solid tumours.[17] The concept of cancer stem cells dates back almost as far as the discovery of somatic stem cells in the haematopoietic system, the skin and gastrointestinal tract crypts.[18,19] There is in vitro evidence that demonstrates that cell populations that are not stem-cell-like can give rise to increased proliferative capacity when an oncogene is artificially over-expressed.[20] This suggests that stem cell populations may not be the only cells capable of undergoing transformation, but that TA cells can also form tumours.[20] However, it is unclear if these cells are immortal and/or have the capacity to develop full-blown malignancy. It is possible that these cell types can only form benign conditions, which would suggest that they lack the proliferative potency required for malignant disease. Importantly, the results of these experiments do not deny the notion of stem cells being the precursor cells targeted during carcinogenesis, but reveal the further importance to our understanding of completely elucidating the origins of cancer development. Other studies suggest that the make-up of the extracellular matrix has a dramatic effect on the differentiation profiles of cells, and that papillomas can be generated from differentiated keratinocytes.[21] It is not known whether these populations revert to TA cells or stem cells, but it has been revealed that recapitulation of the stem cell niche is important in cancer development.[22] So far, p63αΔN and integrin 1β are the most widely reported biological markers that have been demonstrated robustly and singularly to identify unipotent stem cells.[21]

2. p63 and epidermal stem cells

Several groups independently identified the third member of the p53 family, *p63*, also known as *p51*, *KET*, *p40*, *p73L*, *p53CP* and *NBP*. p63 was later shown to be crucial in the development of all epithelial tissues.[23] The expression profile of the p63αΔN isoform was demonstrated to represent the holoclone population of cells present within the epidermis.[11] p63 exhibits a rather tissue-specific distribution pattern and is highly expressed in the ectodermal surfaces of the limb buds, branchial arches and epidermal appendages, which are all sites of reciprocal signalling that direct morphogenetic patterning of the underlying mesoderm.[24] Embryonic epidermis of $p63^{-/-}$ mice undergoes unusual processes of non-regenerative differentiation, culminating in a striking absence of all squamous epithelia and their derivatives, including epidermis, mammary, lachrymal, prostate, lungs, gut and salivary glands.[24] p63 is expressed in the ectoderm prior to stratification during embryonic development. As the epidermis matures in normal mammals, p63 becomes confined to the stratum basal.[24] More recently, the most predominantly expressed isoform, p63αΔN, was demonstrated to be a consistent marker of keratinocyte stem cells within the epidermis[11] (Fig. 1(A and B)). It was also revealed that integrin 1β and p63αΔN were markers representing the same cell populations within the human epidermis.[25] These data are of significant interest in epidermal biology due to the consistency of two independent markers representing stem cells populations (~5–10% of the total epidermis), which adds validity to the use of these markers for future studies and applications and justifies further investigations into the possibility of a functional relationship between them that perhaps mediates fate decisions of stem cell populations. One could postulate that p63αΔN may control integrin 1β expression and that, in turn, integrin 1β may regulate signals exerted from the extracellular matrix (stem cell niche) into the p63 transcriptional cascade. It was reported recently that a member of the integrin family, the integrin α3 subunit, is indeed under the transcriptional control of p63ΔN isoforms.[26] This provides a molecular basis for the hypothesis that the p63 family is essential for epidermal–mesenchymal interactions controlling stem cell fate.

The p63 gene locus has several transcription start sites and thereby creates functionally distinct protein isoforms, some of which lack the NH_2-terminal transactivation domain and are thought to function as 'dominant negative' proteins, e.g.

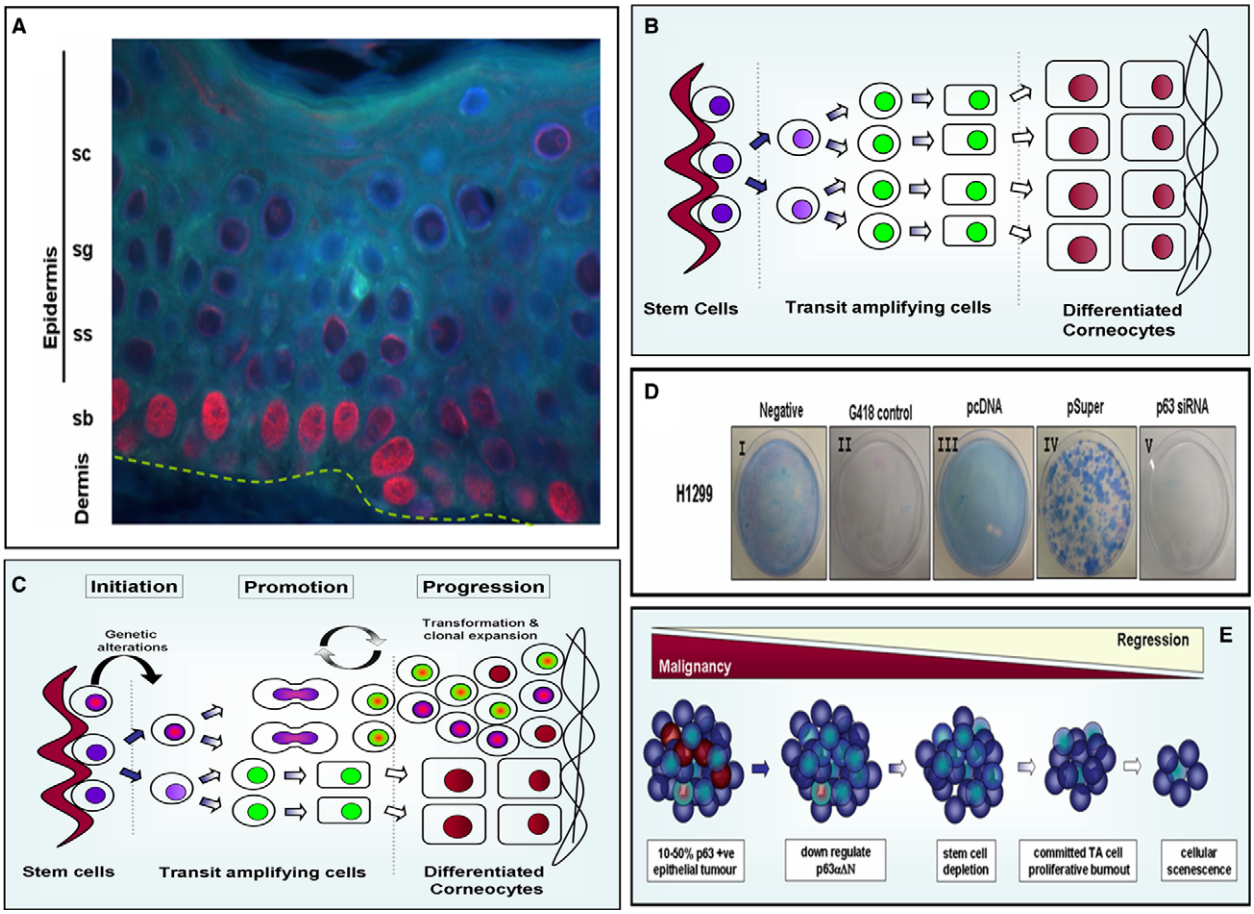

Fig. 1 – p63αΔN is a marker of unipotent epidermal stem cells and a potential therapeutic target that is over-expressed in putative cancer stem cells. (A) Immunofluorescence against p63αΔN in unstressed normal human skin. p63αΔN (red), and counter-stained with DAPI (blue). The dashed line denotes dermal–epidermal border: sb, stratum basal; ss, stratum spinous; sg, stratum granulosum; sc, stratum corneum. (B) Epidermal model of differentiation. p63αΔN-positive 'island-like' populations reside along the basal layer (blue nuclei) attached to the basal laminae (dermal–epidermal border) that express high level of keratins 5 and 18 (K5 and K18). Cell populations that have become committed to a stratification programme at early and late stages express keratin 5 and 14 (K5, purple nuclei and K14, green nuclei) representing transit amplifying (TA) cells that proliferate to develop and replenish the outer layers of the epidermis in co-ordinated fashion. Terminally differentiated corneoctyes are incapable of proliferation and express high levels of keratin 10 (K10) and differentiation marker involucrin that form the cornified envelope that protects the epidermis from environmental exposure (red nuclei). (C) A proposed model of initiation and progression of epidermal tumourigenesis. High proliferation capacity p63αΔN-positive 'island-like' cell populations along the basal layer (blue nuclei) are sensitive to chronic exposure to genotoxic stress. Key genetic alterations in genes such as p53, K-ras, p14^{arf} and epigenetic effects take place within cells chronically exposed to a stress resulting increased risk of damage to stem cells. The selective growth advantages these genetically altered stem cell populations possess, promotes tumour formation. (D) A potential strategy to eradicate cancer stem cells (p63siRNA plasmid originally donated by James DiRenzo, Dartmouth University). High proportions of p63αΔN-positive cells that have become genetically altered stem cells give rise to cancer stem cells, which have been demonstrated to hold the ability to develop the rapid growth of an epithelial tumour. For example, p63 siRNA colony formations assays reveal that p63 depletion can effectively abolish the proliferative capacity of a wide range of epithelial cancer cell types. (I) Control; (II) G418 selection control; (III) pcDNA transfection control; (IV) pSuper siRNA non-sense vector control; (V) p63-specific siRNA treated sample. Pictures taken from tested lung carcinoma cell line H1299 are shown. (VI) Illustration of a therapeutic strategy; p63αΔN-expressing tumours have a high malignant potential. (E) Depletion of p63αΔN does not necessarily trigger apoptosis rapidly, but depletes the ability of p63αΔN-positive cell populations from unlimited proliferation and drives cells into a terminally differentiated state resulting in tumour regression. p63αΔN depletion dramatically affects the ability of many epithelial cancer cells to form a tumour we have tested in vitro.

p63αΔN, p63βΔN and p63γΔN, which block the function of the corresponding full-length proteins. p63ΔN isotypes were shown recently to be able to function as bona fide transcription factors in their own right.[27,28] The reported presence of a second intrinsic transactivation domain (TA2) is thought to be critical for this function.[29] p63 has two transcriptional

start sites generating TA or ΔN isoforms (C-terminal). N-terminal splicing gerantes α, β, γ, isotypes. Therefore, there are at least six p63 isoforms with a complex array of similarities and differences in their structural domains and cellular functions.[30,31] It is becoming clear that the six isoforms of p63 play pivotal roles in the induction of a stratification programme during development, whilst maintaining pluripotent stem cell populations in mature epidermis.[32] A survey of genes up-regulated by p63 shows that p63 can regulate a wide range of downstream gene targets with various cellular functions, including cell cycle control, stress, signal transduction and development.[28] Evidence that p63 is indeed a marker of stem cells within epidermal tissue, and further understanding of how p63 interacts with other factors may be useful in elucidating how stem cell markers are involved in anti-tumourigenic responses, such as cross-talk between the tumour suppressor p53, which has evolutionary functions to prevent and/or eliminate the creation of abnormal cells with the proliferative potential to form tumours.

The development of the epidermis requires an orchestrated progression of events that regulate proliferation and differentiation of keratinocytes. The p63 family has been strongly identified to be the key transcriptional regulator behind such a series of events in keratinocytes; however, it is important to mention that little has been reported with regards to p63 expression in other stem cell populations present in the skin, such as mesenchymal, melanocyte, haematopoietic, neural and endothelial stem cells, which generate more than 25 lineages that contribute to the development and function of the skin.[9] The p63 gene locus at chromosome 3q27–29 bears strong homology to the tumour suppressor p53 and to the related gene, p73. Of the six known isoforms, p63αΔN was demonstrated to be a stem cell marker essential for the regenerative proliferative capacity to develop the make-up of the entire epidermis.[11] p63αΔN is expressed in a tightly regulated fashion with prominent basal epithelial staining pattern. p63 isoform-specific immunohistochemistry has demonstrated that ΔN-isoforms predominate in the basal layers of the epidermis and several other epithelial tissues.[33]

Genetic studies have been used extensively to determine the functional importance of p63 and have shown that p63 is crucial to preserve the regenerative proliferative structures seen in epithelia. $p53^{-/-}$ mice develop normally, but possess an accelerated occurrence of cancer.[34] Mice lacking p63 ($p63^{-/-}$) displayed severe defects in limb, cranio-facial and epithelial development. Additionally, the skin was hypoplastic and was shown to have undergone a program of non-regenerative differentiation.[24] Consistent with the murine phenotype, these disorders are characterised by regenerative failure of epithelial structures. These studies depict that p63 functions to maintain the regenerative capacity of epithelial structures throughout the body. They also support a model in which disruption of p63 expression or activity represents an early step in cellular differentiation.[35] p63αΔN has been found to be over-expressed in several different human cancers, often as a result of gene amplification[36–38] and thus may be important for maintaining the immortal status of cancer cells, clearly marking p63αΔN as a key target in cancer research and, possibly, therapeutic invention. While epithelial progenitors must resist developmental apoptosis, it follows that, under conditions of genotoxic stress, this resistance would be bypassed to allow for the destruction of damaged cells that were potentially transformable due to their attributes of immortality and high proliferative capacity needed for tumourigenesis.[39] p63 and p73 are known to contribute to mediating apoptotic cell kill in response to genotoxic stress in a wild-type p53 background using mouse embryonic fibroblasts.[40] However, TA-p63 isoforms have been demonstrated to be the predominate isoforms involved in mediating apoptosis.[29]

Treatment of keratinocytes with epidermal growth factor results in an increase in p63αΔN expression at the mRNA level, which is abrogated by inhibition of phosphatidylinositol 3-kinase (PI3-K) but not mitogen-activated protein kinase (MAPK) signalling. These results demonstrate that p63 is probably modulated by phosphorylation events that are either stabilising or degrading p63. Regulation of p63αΔN expression by the PI3-K pathway plays a critical role in the survival and proliferative capacity of squamous epithelia.[41] There is extensive information on the regulation of p73[39] by phosphorylation, but little has been reported so far with regards to p63. There has been confirmation that p63αΔN is a phospho-protein in neonatal human epidermal keratinocytes,[42] thereby revealing a whole new area of investigation for the future. Moreover, this report highlights that p63αΔN becomes increasingly transcriptionally active at the $p21^{(waf1/cip1)}$ and 14-3-3σ promotors as keratinocytes undergo differentiation. This is in accordance with the studies of Pellegrini and Dellambra, respectively,[11,43] who demonstrated that 14-3-3σ protein levels accumulate as cells leave the stem cell compartment and RNAi inhibits stem cells from leaving the stem cell niche. A potential mechanism of a p53-regulated protein, such as 14-3-3σ, controlling the activities of p63 in promoting differentiation following stress is compelling. It is important to note that interplay among the p53/p63/p73 family is strikingly shown by multiple-knockout mice, where the classical tumour suppressor activities of p53 are completely lost due p53 being inactive if p63 and p73 are absent.[40] Further discussions of how p63αΔN may impinge strict regulatory mechanisms upon the activities of p53 are given later in this review.

3. p63 over-expression in many tumours: putative cancer stem cells?

Over-expression of select p63 splice variants is observed in many squamous carcinomas, suggesting that p63 may act as a proto-oncogene.[44] The use of various model systems and the study of human disease should continue to lead to rapid advances in our understanding of the role of p63 in development, epithelial cell maintenance and tumourigenesis. The existence of cancer stem cells has been demonstrated in many tumour classes of different tissue origins. A study of human skin cancers revealed basal cell carcinomas (BCCs) showed diffuse p63 expression and squamous cell carcinoma (SCCs) possessed heterogeneous p63 expression with negativity in terminally differentiated squamous cells.[45] All preneoplastic epidermal lesions showed p63 expression in all cell layers.[45] In addition, a correlation between hTERT, survivin expression was seen with p63 expression. By reverse transcriptase-polymerase chain reaction (RT-PCR), it was shown

that p63αΔN is the predominant isoform that is highly over-expressed in cell lines from SCC of the head and neck, confirming immunochemical observations.[46] A correlation between high p63αΔN expression and poor prognosis in HNSCC was reported recently.[47] Protein over-expression in primary lung tumours was limited to SCC and tumours known to harbour a high frequency of p53 mutations. A role for amplification and over-expression has been implicated in lung cancers.[48] Over-expression of p63 in Rat 1a cells led to an increase in soft agar growth and tumour size in mice. These results support the idea that p63 plays an oncogenic role in human cancer.[38] Microsatellite analysis revealed that 14 of 26 (54%) primary head and neck squamous cell carcinomas (HNSCCs) had allelic imbalance in at least 1 of the 7 microsatellite loci. However, fluorescence in situ hybridisation (FISH) analysis with a p63 gene probe showed that a majority of HNSCCs had an increased copy number of the locus regardless of allelic status.[37] Despite an abundance of reports of p63 over-expression in many tumours, it is becoming clear that p63 is very rarely mutated in cancers.[11,49] These data exemplify that the stem cell factor p63αΔN is in fact a proto-oncogene, in direct contrast to its family member p53, a key tumour suppressor. Recent data has suggested that the over-expression of p63 and alteration of p53 regulation are not mutually exclusive in melanoma,[50] which perhaps may be predicted if the only functions of p63 isoforms were to counteract p53 transcriptional responses by repression.[51] However, there is evidence to suggest that a selective programme of events in cancer development may exist that protects p63 from mutation but potentiates its expression while actively down-regulating p53 protein levels by degradation or mutation.[52]

4. p63 stem cell regulation and p53 tumour suppression: 'archangels' of life and death

UV radiation emitted by the sun is a major carcinogen (both initiator and promoter) for most skin cancers.[12,53] Pro-carcinogenic effects of UV-light may be blocked by three distinct, but potentially interrelated, cellular responses involving epidermal keratinocyte stem cells, namely (i) DNA repair; (ii) apoptosis; and (iii) senescence (terminal differentiation). One of the most widely studied responses to UV light is for DNA-damaged keratinocytes to be eliminated via apoptosis, a process referred to as 'cellular proof-reading'.[12,54] The primary mediator responsible for removing DNA-damaged keratinocytes in skin is believed to be p53. The so-called 'guardian of the tissue' role for p53 has dominated the field of skin research for 15 years.[13,55–59] It has been reported that all adult human epidermis contains a large number of p53 mutations apparently without detrimental effects.[60] In this study, the only result of certain patches of cell with p53 mutation may be benign lesions. The importance of p53 mutations for such benign cell multiplication and malignant transformation, on the other hand, is unclear, but a possible explanation is the fact that the cells that are being hit do not possess the capacity to form a sustainable lesion. Compact p53 staining patterns were demonstrated to represent a clonal population of cells with mutated p53 that were derived from one transformed cell (Fig. 1(B and C)), demonstrating p53 gene mutations being early events in the sequence from dysplasia to invasive SCC of the skin.[14] Moreover, examples of p53 being inactive in differentiated cells in both normal epidermal tissue and psoriatic conditions where hyper-proliferation is accompanied by hyper-differentiation adds further evidence.[60–62]

The discovery of the p53 family members, p63 and p73, opened up new dimensions into how a complex surveillance matrix exists that plays critical roles in both development and disease. Research advances have clearly shown that p63αΔN is a stem cell marker of epithelial appendages and maintains the virtues of near pluripotent proliferative capacity and self-renewal, a key property most certainly required by unipotent stem cells and exceptionally evident in cancer development. The discoveries that p63 is widely over-expressed in a wide variety of tumours once again leads to a potential target from the p53 family that may be exploited in cancer therapy. Defining evidence is revealing that p63 comprehensively links stem cell fate and regulation to the tumour suppressor p53. Cross-talk between family members p63 and p53, which evidently have distinct roles, does exist in the human epidermis, which tightly regulates stem cell populations within the stem cell niches from gaining irreversible genomic damage and/or development of abnormal keratinocytes that lead to cancer. p63αΔN may lie at a pivotal point, both in the regulation of stem cell fate, the co-ordination and control of the tumour suppressor pathways that in turn have been demonstrated to down-regulate p63 following DNA damage induced by UVB and/or solar simulated radiation, respectively.[63,64] Further evidence of this was seen by observations in our laboratory following clinically applied UVB to normal human skin in vivo, which gave rise to the identification of positive cells that possessed phosphorylated (serine 15, and serine 392 positive) forms of p53 within putative stem cell clusters (island-like) that reside along the basal laminae.[65] These results were distinct from the staining pattern gained using antibodies that detect total levels of p53 as previously reported by many groups including ourselves.[65–67] These data represented some of the first studies to identify certain cell populations to be promoted to become cancerous using stem cell markers and p63 reagents.[7]

Activation of p53 by UV radiation plays a role in the suppression of development of human skin cancer, and mutation of the CK2 phospho-acceptor site in mice transgenes increases UV-induced skin cancer formation.[68] Using human skin as a model to identify physiologically relevant modulators of p53 phosphorylation, it has been found that there is a selective induction of p53 phosphorylation in the p63αΔN-positive basal layer in UV-damaged skin. In vitro differentiation of HaCaT keratinocytes depletes p63αΔN protein, reduces steady-state ataxia telangiectasia mutated (ATM) kinase protein levels, and results in attenuation of ATM-dependent p53 phosphorylation at at least three distinct key regulatory sites. Depletion of p63αΔN protein using RNAi similarly reduces the levels of ATM protein and results in a reduction in the steady-state levels of p53 phosphorylation at these sites (serine 15, 371/6 and 392, respectively). p63αΔN transfection stimulates p53 phosphorylation, and this coincides with enhanced stimulation of the ATM promotor and elevation of endogenous ATM mRNA (Fig. 2(A)). This suggests that the ATM pathway can be selectively

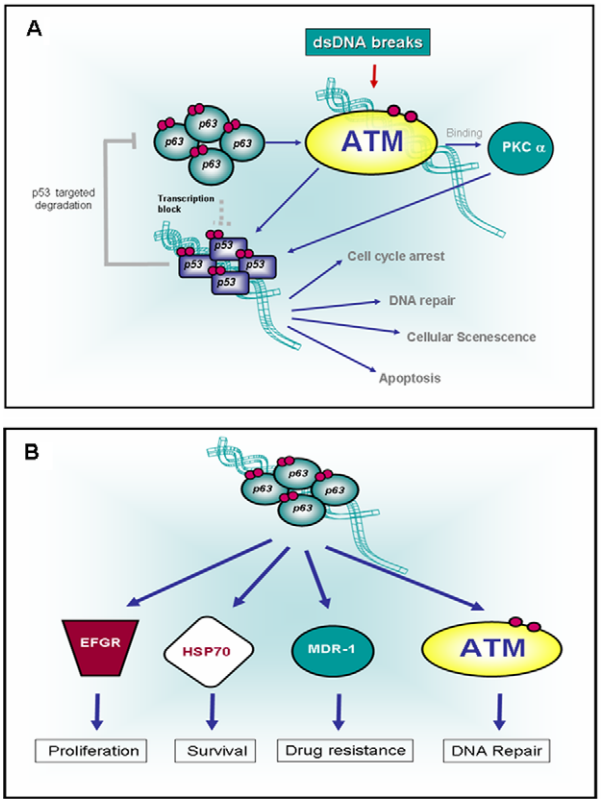

Fig. 2 – p63 can maintain regulators that are important for stem cell protection and which may be inherited and over-expressed by transformed cancer stem cells. (A) The activation of p53 is primarily brought about by specific phosphorylation at key sites within the protein. Interestingly, N-terminal Ser-15 (ATM), Ser-20 (Chk2) and Thr-18 (ATR) and C-terminal Ser-271/276 (PKC) Ser-392 (casein kinase II) have been implicated in the activation of p53 via ultraviolet radiation-mediated damage. Phosphorylation promotes the dissociation of mouse double minute-2 (MDM2), and the formation of stable p53 tetramers required full transcriptional activity. Interestingly, the master kinase ATM seems to amplify its actions of activating p53 after genotoxic insult by recruiting other stress responsive kinases. The recent finding that PKCα mediated phosphorylation of p53 is regulated by ATM further demonstrates the importance of ATM and the data that the latent ATM levels (fully activated by DNA damage) can be transcriptionally controlled by the stem cell marker p63αΔN in keratinocytes. (B) p63αΔN has been reported to transcriptionally control key factors involved in growth (EGFR), survival (HSP 70), drug resistance (MDR-1), and DNA repair (ATM). These markers have been documented to be over-expressed in a large number of solid epithelial tumours, but the question remains as to whether there is a relationship with the expression of these markers in putative stem cells populations?

maintained by p63αΔN protein and identify a physiological model with which to identify novel signalling pathways that stringently protect basal cell keratinocyte populations in response to DNA damage (Finlan and colleagues, CRUK Centre Edinburgh University).

It is becoming clear that the p53 family as a whole are akin to 'archangels' of the epithelium, which are key regulators of specific biochemical lineages that require co-operation, both to shape the destinies of many tissues vulnerable to transformation and in maintaining overall homeostasis by both promoting life and mediating elimination of irreversibly damaged stem cells by death.

The functional discoveries of the other forms of p63 reveal that they possess functionality as both transcription factors and attenuators themselves; however they are not thought to be essential for maintaining proliferative capacity, but are probably essential for effective differentiation processes and apoptosis. Aberrations in their activity may also be involved in the creation of a cancer stem cell, but this remains to be reported. Certainly, several p63 isoforms have been demonstrated mildly to induce p53 regulated genes themselves and also to possess the ability to block p53 transcription by residing on p53 promoters by their mild transcriptional activities of these genes (promoter docking). This reveals other key events that may be critical in determining the overall cellular outcome of p63/p53 interplay at promoters depending on the cell type, stage of differentiation, and exposure to stress.

5. Strategies to eradicate cancer stem cells

New insights into the exploitation of targeted therapy towards populations of cancer cells that maintain the ability to form a cancer are being investigated worldwide. One of the hallmarks of cancer that is coming to light is that many tumours possess a population of cancer cell progenitors that repopulate the tumour mass as proportions of the cell populous undergo a differentiation process and subsequent apoptosis (known as 'cancer cell turnover'). Cancer stem cell progenitors are perhaps of particular importance to future cancer treatments, since they offer a potential target that maybe easier to select for than do most of the current therapeutic targets, which are often expressed on a vast majority of different cells regardless of their differentiation state or tissue type. It is in no way circumstantial that the two most well-documented stem cell markers and effector molecules, p63 and integrin 1β, are rarely mutated in any form of cancer.[21,69] This is in stark contrast to the tumour suppressor genes (often the major focus for cancer treatment), which are often silenced by mutation, proto-oncogene over-expression, and/or promoter methylation. The difficulty with trying to reintroduce wild-type versions of the tumour suppressor genes back into cancer cells is counteracted by the dominant negative mutants intrinsic to the tumour's survival. This contravenes the functions of the wild-type constructs reintroduced by various methods. However, if stem cell survival factors were the targets of therapeutic strategy it would not be through the reintroduction of the wild-type gene, but more through strategies or down-regulation of key survival factors that are often over-amplified and highly expressed in the seeding populations of many tumours. The endpoint of these approaches is likely to be much less complicated to achieve and with lesser technical difficulties. For example, we have used siRNA specific to p63 gene (which selects all isoforms), which shows a great affinity for p63αΔN in many cancer cell types in culture

conditions. Depletion of p63 has a very potent effect on the proliferation rates of tumours when compared with the relevant controls. p63 depletion does not result in direct apoptosis to contribute to these effects, but in cancer stem cells (which are highly p63αΔN-positive) undergoing a differentiation process due to loss of p63αΔN. This culminates in a dramatic effect (over 95% efficiency) on an array of epithelial cancer cells by eradicating the ability of a population of cells to repopulate the plates over a 14 d selection process (Fig. 1(D)). Although these data do not completely describe the niche a tumour possesses in humans or the mammalian system, they do offer increased knowledge in an area of research that should be further investigated. In support, the use of tamoxifen, which targets oestrogen receptor (ER)α-positive breast cancer cells in humans has for many years been a first-line therapy that has shown significant efficacy. This therapeutic strategy is similar to depletion of stem regulation factors, but instead tamoxifen impinges upon the growth stimuli required by some tumours, which are likely to be close to the cancer stem cell in their origin, but it does not eradicate cancer stem cells.[70] A relationship between ERα-positive and putative stem cell marker msi-1 positivity has been demonstrated in the normal mammary gland.[71] The signalling growth hormone receptors are likely to signal up a hierarchy of control mechanisms to induce its effects. It may be that p63 is higher up in the grand control mechanisms of stem cells of epithelial tissues, and is selectively over-regulated in cancer stem cells, thereby providing another example of a mechanism that provides these cells with a selective growth advantage over normal cells, as is often mentioned of cancer cells.[72]

The notion of stem cell factor depletion would be akin to targeting the virtues of the 'queen bee' of an aggressive hive and preventing any other bee from assuming the role and attributes of the queen. It would be plausible that the demise of the 'killer' bees would occur due to a lack of capacity to propagate (Fig. 1(D)VI). It is an interesting concept, but much more rigorous mapping of the functionality of p63 and the governing effects it may have on a host of responses, such as regulation of tumour suppressor genes, cell cycle control factors (such as cyclin D1) and DNA replication. A bona fide target, which could be effective in gaining control over the fate of malignancy and eventual metastasis without the high doses of radiotherapy or chemotherapy currently employed, is much needed. To regress tumour size using therapies that are known to induce substantial DNA damage and cell death as a mechanism to counteract cancer is inadequate, and eventually results in promoting the clonal expansion of chemo-resistant cancer cells. Selectively targeting epigenetic factors known to be important in the regulation of the hierarchy of a tumour maybe a successful strategy to avoid mediating further genetic damage.

A complication of targeting stem cells is the potential for non-specific cell kill of normal stem cells. Although much research remains to be done in this area, it may be that the large surface area of stem cells throughout whole tissue networks is likely to be able to tolerate stem cell factor depletion, such as p63 targeted by siRNA oligonucleotides, if the therapy was targeted. It can be speculated that, if therapeutic investigations into the use of siRNA targeted at p63 were pursued, different strategies of attack would have to be studied intensively in order to rule out any unforeseen detrimental effects of stem cell targeted therapies in cancer. However, the success of the kinase inhibitor imatinib gives great hope for the future of these approaches. Imatinib is a potent kinase inhibitor against the kinase BCR-abl that has been found to be a truncated kinase highly specific to tumour-initiating cancer stem cells of the haematopoietic system. These BCR-abl(+) cell populations are known to be a major aetiological factor in the development of chromic myeloid leukaemia (CML) and have been targeted effectively with successful and dramatic effects on survival rates of patients treated with imatinib (epigenetic) compared with classical DNA-damaging chemotherapy.[73] Moreover, the issue of de-differentiation may also be postulated to create complications for such therapeutic approaches, but differentiated cancer cells may become the key target of the therapy if the re-express p63αΔN. Little data exists in this area, as the field of cancer stem cells is still in its infancy; it is hoped that these issues will be addressed for solid tumours in the future.

6. Conclusion

From the data provided here one can propose key questions to be addressed:

- Do the selective pressures that occur during cancer development require p63αΔN in all cases of epithelial cancer progression?
- What effect does down-regulation of p63αΔN have on cancer cells that have high levels of p63?
- Can cancer cells compensate for loss of p63αΔN expression under the selective pressures they face?

One could say with confidence that the answers will be complex and will not be the same for every form of cancer. However, the general validity of the concept of cancer stem cells is now starting to be demonstrated robustly. It has been proposed by many leading scientists that current cancer therapies are capable of eradicating both TA cells and terminally differentiated cells, but it is becoming clear that cancer stem cell populations possess key factors inherent in normal stem cells that act to protect these cells, such the multi-drug resistance factor 1 (MDR-1),[74] heat shock protein 70 (HSP70),[75] epidermal growth factor receptor (EGFR)[41] integrin family members[26] Perp[76,77] and ATM (Finlan and colleagues, CRUK Centre Edinburgh University) (Fig. 2(B)). These factors are key players in chemotherapeutic resistance and uncontrolled proliferation. These key proteins have received attention as potential drug targets in cancer, and drugs are in development for cancer therapy for various tumour types. The possibility remains that p63αΔN may be a reason why these factors are present within tumours that resist chemotherapy, thus depletion of p63αΔN may not only reduce their proliferative potential but also potentially disarm tumours of their key mechanisms of resistance (Fig. 1(E)).

There is a theory that current chemotherapies culminate in the promotion of selective resistance to therapy, providing a possible explanation as to how and why tumours become resistant to therapy and more aggressive. The point to bring to the discussion on p63 and cancer stem cell control is that

p63 is highly expressed in many epithelial tumours and very rarely mutated in cancer. It may therefore only require down-regulation to remove the proliferative capacity associated with p63 expression in cancer and to eradicate cancer cells. This may be easier than the reintroduction of a wild-type tumour suppressor pathway, which is likely to be counteracted by the dominant negative mutant tumour suppressors present in tumours. Further understanding of the origins of cancer stem cells and investigations into the control of normal adult tissue stem cells are important, as a successful therapy depends upon targeting the cells within a tumour that drive the overall growth of cancer. The key to developing effective therapeutic targets lies ultimately in the identification and characterisation of biological determinants of stem cells and investigations into the roles they play in cancer. These discussions aim to reveal alternative approaches with which to compare and contrast the hopes of therapies based on cancer stem cells with those of a coarse nature, such as chemotherapy. The title of a recent review on leukaemia is an elegant axiom for consideration here: 'punish the parents, not the progeny of cancer'.[73]

Conflict of interest statement

None declared.

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