Implication of stem cells in the biology and therapy of head and neck cancer

Abstract

The progress which has been made in the therapy of patients with head and neck cancer in recent years mainly concern the HPV associated HNSCC and the quality of life. The overall survival of patients carrying non HPV associated HNSCC during the last thirty years has not experienced any significant improvement and must be referred to as static [1], [2]. The problem of the illness remains unchanged in the frequent and poorly controllable relapse situation. The locoregionally originating tumours or lymph node metastases show a considerably poorer response towards current therapies. Likewise for a number of patients a formation of distant metastases seems to develop during the course of the illness. Those distant metastases are also therapeutically rather difficult to control. Therefore the mortality of the non HPV induced head and neck cancer remains unchanged.

The term "stem cell" describes the entity cell, which acts as a reservoir for new cells in order to replace defective or necrotic cells. A fundamental characteristic of stem cells is the constant ability to multiply into different type of cells, which subsequently do not proliferate.

With the insight of new knowledge within the regenerative medicine and the ability of stem cells of self regenerating proliferation and their multipotency in the differentiation, the origin of cancer attains a new distinction. If you look on the tumour as a malignant wound it becomes obvious, that the regeneration or the composition of additional tissue depends on the presence and differentiation of stem cells. The wound healing, which is a regeneration of tissue depends not only on stationary stem cells. In fact stem cells are attracted for "homing" in the defective areas by despatch of various messengers, which then form and replace the vascular tree or other tissue [3], [4].

Next to those stem cells, which functionally help to form tumour tissue, a small entity of "real cancer stem cells" in solid tumours is expected. Those occur in tumours and they have typical stem cell characteristics like self-regeneration and the potential of differentiation and are potentially responsible for tumour growth. With their ability of self-regeneration they would have the ability to form a complete tumour out of every single cell. That tumour would histologically look like the tumour those cells initially originated from. Of particular interest regarding those currently still elusive cancer stem cells is their resistance towards current therapies like radiotherapy or chemotherapy. Those insights now get a completely new meaning in tumour biology: Does a cancer stem cell exist, which is able to initiate and keep up tumour growth despite all possible therapeutic interventions?

This presentation will outline the current views regarding cancer stem cells in non HPV associated HNSCC and it will highlight problems, which are currently researched on. The objective must be to understand the biology of those cells in a way that make an extended range of therapeutics possible. A therapy, which specifically targets cancer stem cells, could improve the chances of recovery.

Keywords: head and neck squamous cell cancer (HNSCC), stem cells, cancer stem cells, CD34, WNT pathway, self renewal

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1. Tumour infiltrating stem cells

Currently the degree of contribution of stem cells towards the regeneration of cancer is widely not understood. Resulting of my own observations and analysis of literature it becomes clear, that HNSCC is infiltrated by a large number of stem cells [5]. For many of those stem cells it unclear where they originate from. The source could be either in the bone marrow or in peripheral tissue [6], [7]. Using the example of the CD34 progenitor cells described below the implication of such infiltrating stem cells will be highlighted, whereby far more cells carrying stem cells markers are present in HNSCC [8].

1.1 Infiltrating CD34 progenitor cells in development and therapy of HNSCC

Over the last years it became obvious that adult stem cells and their precursor contain a highly therapeutical potential. Beside their usage within the regenerative medicine there are increasing indications for a promising usage within oncology [9]. A number of studies could demonstrate that CD34$^{pos.}$ cells are mobilised from the bone marrow into the bloodstream by GM-CSF which is produced by head and neck carcinomas [10], [11]. Furthermore the CD34$^{pos.}$ cells are chemotactically attracted by the head and neck carcinoma by means of VEGF [12]. The possible biological background is many-faceted; stem cells can be involved in neoangiogenesis in order to improve the deficient blood supply in the tumour [4], [13]. Furthermore it seems thinkable, that the tumour, based on the deliberations in terms of a tumour stem cell, promotes the differentiation of the entering CD34$^{pos.}$ cells in tumour cells.

It has been demonstrated that hemopoietic, mesenchymal and endothelial progenitor cells from the bone marrow play a central role in the synthesis of the tumour stroma and in the neoangiogenesis [9], [14]. By means of genetically modified stem cells antitumoural effects in animal models had been already achieved [15], [16], [17]. In addition to it the elucidation and usage of tumour specific stem cell recruitment opens the possibility to induce efficient graft-versus-leukaemia effects in the context of allogenic stem cell transplantations. The genetic modifications of those cells could, in the context of autologous stem cell transplantations, induce antitumoural effects without damaging the normal tissue. Moreover a broad clinical basis for a safe clinical application of those cells exists, because a bone marrow transplantation/stem cell therapy after myeloablative treatment is, for many years, an inherent part of therapy protocols. Additionally those cells are relatively easy to isolate, to multiply and can be genetically modified transiently or permanently in many ways. Hence the expansion of knowledge of those cells within oncology is crucial for the development of new HNSCC therapies [3], [11], [18], [19], [20], [21], [22], [23], [24], [25], [26], [27], [28], [29], [30].

In addition more recent studies have shown, those stem cells develop potent immunosuppressive characteristics under the influence of the tumour environment. It was shown, that T cells obtained from a head and neck carcinoma are significantly limited in their ability to secrete IL-2. However, if one removes the CD34$^{pos.}$ cells from the cell lysates, a distinctly higher IL-2 secretion manifested. On the other hand adding additional CD34$^{pos.}$ cells had the same effect and lead to a significant suppression of the IL-2 secretion [31].

Furthermore it was shown, that the response rate of T cells obtained from a head and neck carcinoma to a IL-2 stimulus in an autocrine stimulation sense is permanently restricted. While a normal T cell population reacts to an IL-2 stimulus with a high proliferation rate, so limited is, on the contrary, the beneficent reaction under the influence of CD34$^{pos.}$ cells. The response rate to such a stimulus is lowered. In the same way, however, the response rate to an IL-2 stimulus can be normalised by removing the CD34$^{pos.}$ cells from the cell lysates [31]. This immunosuppressive influence of those cells is apparently moderated via secretion of TGF-b and other cytokines via CD34$^{pos.}$ cells. In further studies the above-mentioned effects of the CD34$^{pos.}$ cells have been averted by means of antibody based blocking of the TGF-F receptors [31], [32], [33] (Figure 1).

The reflection was deduced from the research results mentioned above, which is to bypass not only the immunosuppressive characteristics of the CD34$^{pos.}$ cells via directed differentiation, but also to influence via directed differentiation their characteristics as a directed immune response against the tumour [34]. In this context those studies are of great interest, which could demonstrate, that CD34$^{pos.}$ cells obtained from patients with head and neck carcinoma can differentiate to dendritic cells [35], [36], [37]. In present tests to recruit dendritic cells as an antitumoural immunotherapeutic, often the number of the cells to be recruited represents the limiting factor. Based on the fact, that a peripherally increased level of CD34$^{pos.}$ cells could be verified on patients with head and neck cancer, this could represent an elegant and promising new approach in terms of an antitumoural immunotherapy [38], [39], [40].

2. Cancer stem cells

Cancer stem cells (CSC) are characterised by specific markers, which very much depend on the origin of the CSC's surrounding tissue. Cancer stem cells are initiating and sustaining carcinomas, because they are self-regenerating and remain resting in most carcinomas. Other cancer cells make up the largest part of malignant cells; however, they do not contribute to the malignancy of the illness to the same extent. It is of particular interest, that some cancer stem cells resist the common chemotherapies and radiotherapies and show a low immunogenicity. This resistance could explain why tumours vanish initially after such therapies mentioned above, but often occur
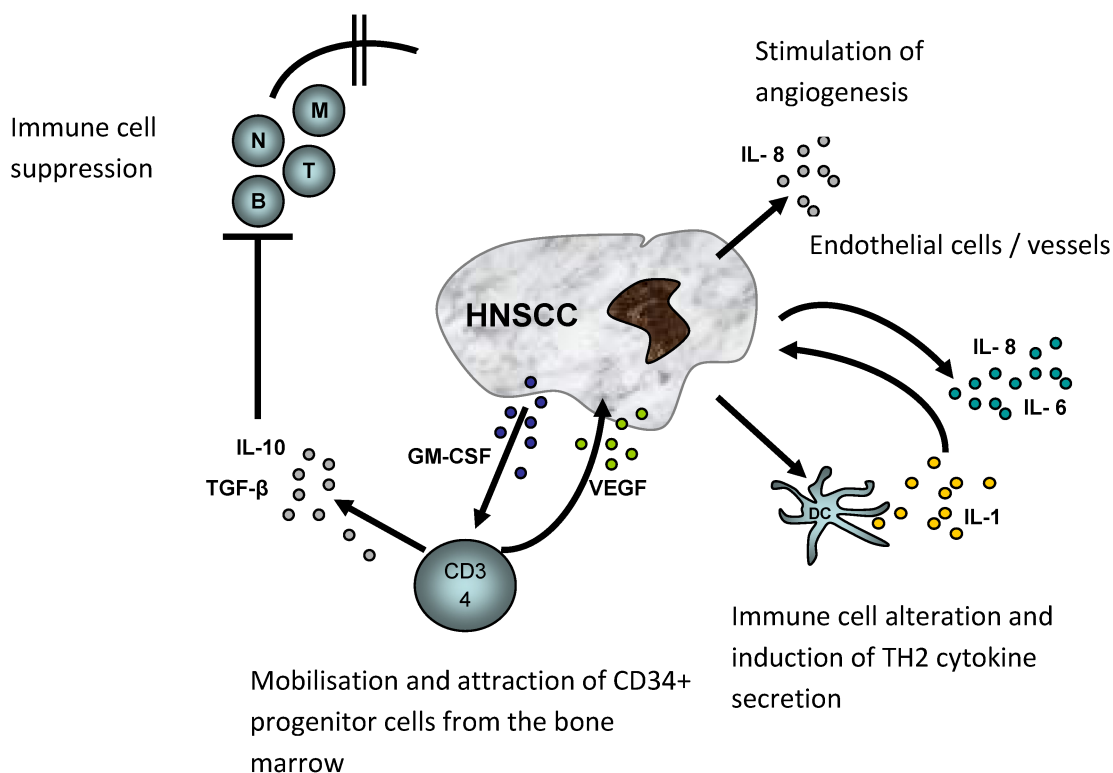
Figure 1: Stem cells in the tumour: The role of CD34- progenitor cells

again later. A therapy, which specifically targets cancer stem cells, could improve the chances of recovery. It is widely believed that the present therapies target the "normal" tumour cells which form the major part of the tumour, but it is also believed, that new concepts must be developed, which reliably eliminate the considerably more resistant cancer stem cells [41], [42]. For this purpose a large number of existing data originate from studies of different cancerous entities, nevertheless there is a large number of data already, which were verified on HNSCC.

2.1 Definition and characteristics of cancer stem cells

From healthy stem cells we know their function of self-regeneration and their ability to differentiate into cells of various tissues. The present findings about cancer stem cells demonstrate that they extend those abilities possessed by normal tissue stem cells [43], [44]:

- Unlimited self-regeneration
- Differentiation into various cell types
- Loss of control of proliferation
- Initiation of malignant tumours on a single cell basis via symmetric proliferation
- Expression of cancer stem cell specific markers

Thus the tumour possesses at least two entities of cells – the tumour initiating cancer stem cell and those cells (non tumorigenic cells) originating from cancer stem cells, which have lost their ability of self-regeneration [43].

2.2 Source of cancer stem cells

The present theory of formation of head and neck cancer can be summarised as follows: Caused by a questionably genetic disposition during a chronic inflammation caused by permanent tobacco and alcohol abuse, mechanic irritation or viral infection, a spontaneous accumulation of various genetic alterations develops leading to a manifestation of a malignant phenotype. Then clonal divergence and selection lead to a formation of a carcinoma. This accumulation of genetic alterations can appear synchronously or metachronously in the area of the entire inflammation of the exposed mucosa which leads to the so called "field-cancerization" [45], [46].

If one carries over the afore-mentioned to the present assumption of the genesis of cancer stem cells, certain parallelisms can be verified, whereby the formation of cancer stem cells is not conclusively clarified for any tumour entity. Currently three hypotheses are discussed [47].

1. Source of "normal" stem cells with cancerous phenotype.
2. Source from differentiated cells with oncogenic mutation (Return to self-regeneration and multipotency)
3. Fusion of stem cells with tumour cells (much discussed with the formation of bone metastases)

Whatever mechanism for the formation of a tumour stem cell one presumes, the offspring cells will retain the signalling pathways and characteristics of the source cell
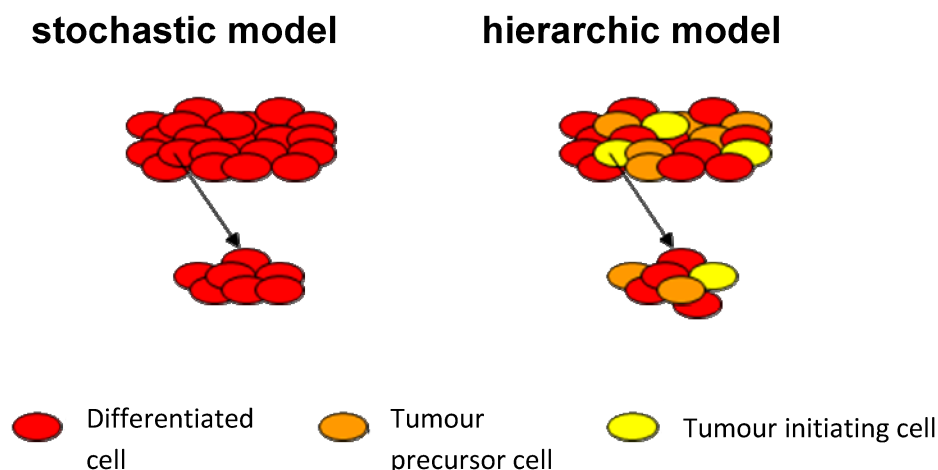
stochastic model   hierarchic model

Differentiated cell

Tumour precursor cell

Tumour initiating cell

Figure 2: The formation of HNSCC: stochastic vs. hierarchic model

they originated from, although many of the signalling pathways are downregulated and therefore inactive.

2.3 Stochastic or hierarchic expansion of tumours

Currently two models describing growth behaviour are being discussed and compared. The *stochastic model* describes the up to now pursued thesis, that there would be one "mother cancel cell", which stochastically proliferates by entry into the cell cycle, whereby each cell within the tumour possesses the same ability to promote tumour growth. Therefore each cell is equally potent to initiate a tumour. The heterogeneity of the tumour is explained by spontaneous shifts in the phenotype (Figure 2).

In contrast to the above is the *hierarchic system*, which splits the cells of a tumour in at least two entities: The tumour initiating cancer stem cell and its differentiated offspring with various degrees of maturation, which lost, however, the ability to initiate a tumour [48], [49]. It is the subject-matter of current research to what extent the heterogeneity of tumour cells within a tumour can be ascribed to those various degrees of maturation. In theory one CSC would be sufficient to initiate a tumour, which develops the same histological phenotype as the tumour, from which the CSC originated from (Figure 2).

As far as HNSCC is concerned it has not been finally clarified which model is valid. One can find arguments for a rather evolutionary formation of a carcinoma, but many things can be explained with differentiation. Therefore there is much to be said for the hierarchic system. Thus, malignant cells within the vicinity of the carcinoma have the same biological behaviour, which can be explained rather with differentiation than with evolution. Furthermore the metastases showed the same heterogeneity than the primary tumour albeit without being exposed to the same DNA damaging noxa.

The quantity of CSC which is needed to develop a carcinoma of identical origin varies among the tumour entities are determined by replantation studies in the mouse. Currently the quantity fluctuates between 10^3 and 10^7 cells [48], [50]. The reason certainly lies in the purity of the isolated and defined as CSC cell population, which can be classed as low for HNSCC due to non existing markers.

2.4 Surface markers for characterisation of cancer stem cells in HNSCC

The definition and isolation of CSC is subject to the problem, that many markers are necessary, which are also expressed in ordinary cells. It is the subject-matter of current research to identify a clear definition and additionally to identify a clear definition of particularly invasive subgroups where applicable. Currently the hereinafter characterised markers are used; more are being tested.

2.4.1 CD44

The isolation and characterisation of recent CSC in HNSCC revolved around the marker CD44 [50]. CD44 is not sufficient, because as a glycoprotein of the cell surface it takes part in many cell-cell interactions, cell adhesion and migration. In humans CD44 is present in a plurality of splice variants, which already have been linked with cell transformation and tumour progression before the discovery of CSC. Thus, the CD44h is the receptor for hyaluronic acid and therefore it is a relevant connecting link in the regulation of extracellular matrix interactions. CD44v6 was linked with the progression of laryngeal carcinoma but CD44 and CD44v6 are also expressed in many benign tissues of the aerodigestive tract. The quantity of the real CSC cannot be determined alone by the measurement of CD44. Furthermore CD44 is expressed in all stem cells of hemopoietic origin. A combination of lineage markers (CD2, CD3, CD10, CD18, CD31, CD64 and CD140), which must be negative, is mandatory [51], [52], [53]. Even the combination of CD44+lin- leads to a selection of cells, which requires a very high number of cells in the tumour induction of the mouse, thus, it does not represent an aggressive or well-assorted popu-

lation. It is characteristic, that the majority of the stable entrenched HNSCC lines are also severely CD44+lin- [52].

2.4.2 CD24

CD24 is a cell adhesion molecule, which is expressed on the surface of many B cells and in differentiating neuroblasts. It encodes a sialoglycoprotein, which is expressed in many mature granulocytes and B cells and in CSC as recently was shown [54].

2.4.3 ALDH1

Only recently Aldehyde Dehydrogenase 1, a member of the cytosolic enzymes, which catalyses the oxidation of the aromatic aldehydes in carboxylic acids, was verified as a potential marker for CSC in HNSCC [55], [56]. In other cancer entities it could be demonstrated that ALDH1 plays a role in the conversion of retinol into retinoic acid, which is important for proliferation, differentiation and survival of, for example, keratinocytes. The activity of ALDH1 is responsible for the resistance of progenitor cells towards chemotherapeutic substances, because it can break down cytotoxic drugs [57]. ALDH1 positive cells are increasingly identified as an objective of therapeutic measurements [58], [59], [60].

2.4.4 CD133

CD133 is a ubiquitous marker for hemopoietic stem cells, endothelial progenitor cells, glioblastoma, neuronal and glial stem cells. It actively takes part in the change of the plasma membrane [61]. For HNSCC an influence on the expression in CD133 for an increased rate of recidivation and secondary neoplasms was verified [62]. An implication of CD133+ in combination with other markers is not clearly investigated yet [63], [64], [65], [66].

2.5 Surface markers for characterisation of cancer stem cells in HNSCC

Both healthy stem cells and cancer stem cells show a great variety of signal transduction pathways, which are necessary to maintain the stemness (integrity and potency of a stem cell) and other various functions of the stem cells. The following have come into view of current research: Wnt/frizzled, Oct4, Snail, Twist, Sox, Nanog, hCG, GSC, E-cadherin, AFP, GATA-4, HNF-3 beta/FoxA2, PDX-1/IPF1, Otx2, TP63/TP73L, Goosecoid (GSC) and many more others. The implication of those signalling pathways shall be exemplarily illustrated by the Wnt signalling pathway, which is the best researched way with regards to HNSCC.

2.5.1 Wnt signalling pathway

The Wnt signalling pathway is involved in the regulation of most different biological processes like, for example, embryogenesis, cell proliferation and cell migration.

Having different differentiation and transformation processes at the back of one's mind the signalling pathway also takes part in tumour suppression and oncogenesis. The cause of an increased cancer risk often lies in a deregulation as a result of mutations, e.g. breast cancer or colon carcinoma [67].

The team around Roel Nusse discover the Wnt1 protein under the name of Int1. They found out, that mice, in which that protein has been over-expressed, contract breast carcinoma. The Wnt1 protein, which holds a preferential integration site of the mouse mammary tumour virus (MMTV) and therefore has been named proto-oncogene Int1, was responsible for it [68]. At the same time a mutation of the wingless gene (wg) was described for a wingless variant of the common fruit fly drosophila melanogaster. By comparison of the sequences both genes have been associated with each other and finally were named Wnt [69].

Figure 1 illustrates the canonic Wnt signalling pathway. With the help of various cofactors the Wnt proteins bind on the transmembrane receptors of the Frizzled family and via the ß-catenin, *the* central component, the regulation is carried out. By the receptor complex's Wnt signal a stabilisation and subsequent accumulation of ß-catenin in the nucleus is made possible, so that a gene expression of target genes can take place with the help of transcription factors.

Cytoplasmic ß-catenin is subject to a permanent degradation process. Newly synthesized ß-catenin is immediately bound to the tumour suppressor genes "Adenomatous Polyposis Coli" (APC) and axin. The activity and bonding of ß-catenin to those two proteins is increased by the phosphorylation of the glycogen synthase kinase 3 (GSK3). Subsequently ß-catenin is phosphorylated and degraded into serine/threonine by the kinases [70].

Implication of the Wnt pathway for HNSCC

Even before the identification of CSC in HNSCC by Prince et al. in 2007 [50], as early as 2002 it was verified on permanent HNSCC lines which were compared with healthy epithelial cells, that HNSCC lines show considerably increased levels of mRNA for Wnt-1, 7a, 10b, 13 and for Fz-2. A blockade of the Wnt pathway by means of antibodies led to a reduction of the factor LEF/TCF which is required for the transcription of Wnt and reduced the expression of cyclin d1 and the ß-catenin pathway. It also led to an inhibition of proliferation and induced the apoptosis in the HNSCC [71]. Yang et al. [72] could verify that the Wnt/beta catenin signalling pathway suppresses the receptor mediating apoptosis in HNSCC.

In so doing not only the tumour necrosis factor (TNF)/c-Myc-mediated apoptosis, but also the cell detaching mediated apoptosis (anoikis), which is dependant on the receptor-mediated pathway, is averted. The Wnt/beta catenin signalling pathway induced cell detachment in otherwise adherent growing HNSCC and supported invasive growth in Matrigel [72].

Other research groups could demonstrate that cells with over-expressed Wnt signals behave highly invasive in vitro, tumorigenic in vivo and chemoresistant towards Bortezomib and Etoposide [73]. In a study of solid HNSCC an association of high expression of WNT7a and Frizzled 5 mRNA with reduced length of survival became apparent [74]. This could be confirmed with other markers along the Wnt pathway. A new transcription factor Rap1 was identified, which effectuates the stable and stability and nuclear translocation of the beta catenin. Clinically this is associated with more advanced lymph nodes stadia of the illness [75].

3. Epithelial-mesenchymal transition (EMT) in head and neck cancer

The development of a complex organism is based on a network of numerous control mechanisms and a stringent controlled order of events. It initially starts with the epithelial-mesenchymal transition, i.e. the transition of epithelial cells in cells with mesenchymal characteristics. In 1982 this developmental and biological process was first described by Greenburg and Hay [76]. Cell-to-cell contacts are broken up by down-regulation of various adherence molecules like E-cadherin and specific signalling molecules (Wnt/ß-catenin, FGF or TGFß1/BMP) induce the cell migration and the development of mesenchymal characteristics.

EMT triggers changes in the expression, in the intracellular distribution and in the function of various growth factors (TGF-ß, transforming growth factor beta), transcription factors (Snail, nuclear ß-catenin), cell to cell adhesion molecules (cadherins, claudines, occludines), cell-matrix adhesion molecules (integrins), modulators of the cytoskeleton (Rho GTPases) and extracellular proteases (matrix metalloproteinases) [77].

As the transition advances the cytokeratins, which take part in the composition of intermediate filaments, are often replaced with vimentin. Furthermore molecules like fibronectin and vitronectin, which enables the cells with an increasing feature to migrate, are increasingly expressed. Caused by those changes the epithelial cells loose their rounded shape and are now able to migrate through the basement membrane [78], [79], [80].

Those events of the embryonic development are very similar to those cellular processes regarding the metastasis of malignant tumours. There, too, the tumour cells obtain the ability to migrate via a phenotype change. In epithelial tumours single cells can dissociate themselves from the cell colony this way and they can spread through the blood system or lymphatic system and form new tumours in other locations.

The necessary dissociation of single epithelial tumour cells, which is required for metastasis, often has been linked with EMT processes [80], [81]. The loss of cell adhesion structures alone, however, does not define an epithelial-mesenchymal transition and therefore the problem of identifying reliable EMT bio-markers and the further characterisation of developing post-EMT cells remains [82], [83].

As early as 2006 gene expression analyses showed a significant correlation between tumour aggression and expression profiles of various genes in the areas of EMT and cell adhesion [84]. Additionally further correlations between tumour progression and EMT regulation are known.

As a result of an activation of FGFRIIB- tyrosine kinase and successive Src-, Ras and signalling cascades bladder carcinoma cells differentiate to wandering fibroblasts [85]. Furthermore a SNAI1 dependant regulation of various EMT associated genes was verified for colorectal carcinoma cells. Snail suppresses the expression of E-cadherin, which is a cell adhesion protein, and therefore plays an important role in the synthesis of mesenchyme from epithelial cell colonies [86].

In mouse models it was verified for breast cancer, that the progression and the metastasis of regressive tumours was stimulated again by the expression of Snail. The formatting tumours consisted of mesenchymal cells [87]. Furthermore in the aggressive-invasive ductal breast cancer an over-expression of the EMT transcription factor FOXC2 was verified [88]. Therefore a model has been described, in which classic activators of EMT like Snail1 or Twist-1, form cells with mesenchymal characteristics out of epithelial tumour cells, which are called cancer stem cells (CSC). Those cells are able to perform self-regeneration and they are able to promote maturation and multiplication in the remainder of the non CSC tumour cells. In this way they contribute to tumour initiation, tumour progression, tumour invasion and metastasis (Ouyang et al., 2010). Because various signalling cascades like Wnt are involved in the ENT in embryology as well as in the regulation of stem cells, coherence between EMT, stem cells and tumour formation, tumour growth and metastasis is assumed.

To date only singular observations are reported in head and neck cancer giving significance for EMT to single proteins like TrkB, ZEB1, NBS1 or Stat3 [89], [90], [91], [92].

4. Stem cell reservoir and tumour niches

The number of postnatal tissue, in which stem cells or progenitor cells were found, has increased dramatically in recent years. Stem cell reservoirs, so called niches, were shown in many tissues. Those niches feature special conditions and structures, which are necessary for self-preservation and regulation of stem cells. In an in vitro culture many of those cells show an enormous plasticity and they can differentiate into various specialised cell types. Thereby it is particularly remarkable, that the number of stem cell types, to which pluripotent characteristics are attributed to, also increased recently. Those

cells are capable of differentiate into cell types of the three embryonic areas.

There is evidence to suggest that tumour stem cells can revive cancerous illnesses after an initially successful therapy and furthermore can be regarded as the source of metastases. Of great relevance for the preservation of those stem cell characteristics presumably is the so called "stem cell niche", i.e. the immediate neighbourhood of the cell, which is defined both by the anatomic structure and by cellular and non-cellular parameters. The tumour tissue apparently holds such niche properties and it is characterised by the expression of certain surface molecules such as cadherins, catenins or integrins [93], [94], [95].

The surrounding tissue and the supporting extracellular matrix are of extraordinary importance for the formation and preservation of a tumour. They rule its identity and its cellular behaviour. If, for instance, ordinary somatic cells are removed from their "niche environment" and cultivated, often retrodifferentiation processes become apparent [96].

By implication it became apparent in transplantation experiments with stem cells showing already malignant degeneration caused by oncogenic mutations, that they do not induce tumour growth in a healthy niche. However, if healthy stem cells were transplanted in pre-damaged tissue, tumours emerged from them. There is much to be said for, that the niche is playing a role in the last steps of a malignant degeneration [97]. One of the mechanisms through which niche factors influence the destiny of cancer stem cells is the control and decision over the proliferation of symmetric (two identical cells) vs. asymmetric proliferation (one identical and one differentiated cell). Factors secreted by the tumour stroma influence the stem cell niche of the tumour microenvironment and provide a suitable setting for tumour expansion. It is entirely not understood by which stimuli and for what reasons dormant cancer stem cells are being re-activated after years in their niche and long after a cancer therapy.

5. Therapeutic implications

For HNSCC only a few papers regarding CSC therapy strategies are available. Taking studies on other cancer entities and the model of hierarchic tumour initiation into account the following order of events must be adopted (Figure 3). The heterogenic tumour will be treated with conventional means. During the surgical intervention residual cancer cells remain in the incisal margin, in the neighbourhood of the tumour and in the adjacent tissue space; those will be treated postoperatively or primarily with combined or primary radiotherapy. The heterogenic tumour's differentiated tumour cells will be destroyed. The considerably chemo- and radio-resistant cancer stem cells remain; because of their stem cell characteristics they are able to generate a tumour, which histologically matches the tissue from where it originated from (Figure 3).

This model further emphasizes the immense implication of safe margins during surgical measures and demonstrates that the objective of future therapies must be the development of specific drugs against the cancer stem cells of a tumour, which remain after the removal of the tumour bulk via conventional therapy treatments. Studies will be required which consider the condition of cells like, for example, the resting cell cycle phase G0, abilities of self renewal, the ability to pass cytotoxic substances out of the cell and many other mechanisms of therapy resistance.

Initial approaches to treat CSC in HNSCC are emerging from the study of such mechanisms. However, none of those studies can be regarded as even close of a clinical-translational realisation. Similar to other immunotherapeutic approaches the biggest problem lies in the selective reachability of cancer stem cells, because they use the same mechanisms, on which the healthy stem cells depend on, too, and this makes a specific targeting difficult.

The chosen therapy approaches to date have only been tested in vitro or in the mouse model.

Self renewal cascades

Until now the targeting of self renewal cascades have been tried using different techniques like on the above-mentioned Wnt/Frz-system. It was verified on permanent HNSCC lines which were compared with healthy epithelial cells, that HNSCC lines show considerably increased levels of mRNA for Wnt-1, 7a, 10b, 13 and for Fz-2. A blockade of the Wnt pathway by means of antibodies led to a reduction of the factor LEF/TCF which is required for the transcription of Wnt and reduced the expression of cyclin d1 and the ß-catenin pathway. It also led to an inhibition of proliferation and induced the apoptosis in the HNSCC [71]. Goto et al. could identify RAP1 as a factor, which increases the nuclear translocation of ß-catenin, the core element of the Wnt signal. An inhibition of RAP1 via small interfering RNA (siRNA) could reduce the translocation, lower the expression of matrix metalloproteinase 7, a transcription of catenin, and thereby functionally lower the invasiveness of HNSCC [75]. A regulation via inhibition of RAP1 is a possible approach in immunotherapy, because high levels of ß-catenin are associated with advanced tumour stages.

But also other self renewal cascades have come into view of therapeutic deliberations. Thus, Chen et al. have found a better response rate of CSC towards radiation by the use of siRNA transmitted silencing of BMI, which is an essential protein of a self renewal cascade [59]. In the same way Snail could be silenced. Snail is believed to be one of the triggers of EMT, which actively transforms epithelial cells into migrating mesenchymal cells and in doing so actively suppresses the expression of E-cadherin, desmoplakin and cytokeratin.
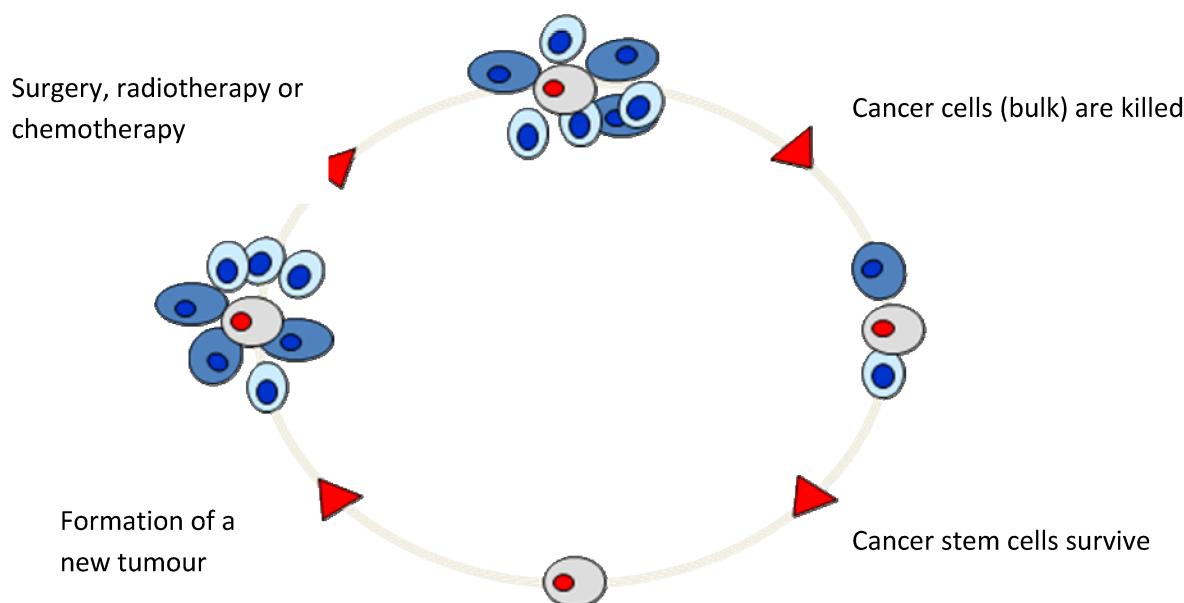
Figure 3: Circle of therapy resistance induced by CSC

T cell therapies and the implication of malignant tissue

As early as 2007 it could be verified, that ALDH1 is an immunogenic epitope, which can be recognised by CD8+ T cells. With all the limitations a T cell response implicates in HNSCC patients, an antigen-specific immunotherapy towards the epitope of ALDH1 seems possible [58].

In the future there will presumably be a stark change in thinking throughout the tumour therapy caused by the results of research. After all the carcinoma cells themselves were in the limelight of most therapy models. In recent years, however, it proved to be true how important the tumour stroma is for the formation, sustainability and also for the tumour eradication. In a mouse model tumours were used, in which the cancer cells expressed a tumour antigen to a lower extent only. When highly specific T lymphocytes were then administered in the mouse against this tumour antigen, a tumour decrease came along with it, but the tumour recovered quickly, because the non antigen carrying tumour stroma had not been attacked and individual cancer cells, which did not express the antigen anymore, could grow further. If, however, the tumours have been treated with radiotherapy or chemotherapy before the T cell transfer, complete tumour eradication occurred. Caused by radiation and chemotherapy also a release of tumour antigen occurred, which was absorbed by the tumour stroma and expressed to its cells. Then the transfected T cells attacked both the antigen carrying cancer cells and the tumour stroma. As a result the cancer cells, which lost the antigen, were also killed, because their subserving tumour stroma had been killed by the T lymphocytes [98]. However, the role of the tumour vessels for the optimal migration of T cells is very interesting in this context, too. Caused by G-protein signalling 5 (RGS5) a reduced maturation of pericytes, which are important for the formation of normal vessels, occurs in tumour vessels. The tumour vessels therefore show distinct leakages for plasma proteins and also cause a worsened oxygenation of tissue. Interestingly those vessels also act as a stark barrier against the migration of T lymphocytes into the tumour, whereby the underlying mechanisms are not really understood. However, if the G-protein signalling 5 was blocked, the pericytes could mature fully and the tumour vessels became apparently more stable. This resulted in an improved oxygenation of the tumour, but it also resulted in an enforced T cell migration, which, in return, could attack the tumour tissue [99].

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