Emerging Strategies against Head & Neck Cancer – A Review

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Abstract: *Cancer is attributed to be the second most common cause of morbidity and mortality in the world today after cardiovascular problems. Oral cancer comprises of a significant component of the global burden of cancer with an annual incidence of over 300,000 cases. Though efforts have been directed to tackle and curb the spread of cancer; little progress has been made in the regard and it continues to pose a threat. Numerous study methods have come to foray assisting and paving way to arrest cancer. Several new stream of drugs targeting molecular signalling pathways like those of growth signal transduction machinery in cancer cells, processes involved in cellular invasion and metastatic spread, apoptosis and the cell cycle, and tumour-related angiogenesis. Other approaches like tumour-specific antigens, targeted poisons, immunotherapy, gene therapy, telomerase therapy, DNA damage repair and nanotechnology have also come to horizon. With the advancements, in the fields molecular and biochemical analyses it has been proposed that drugs can alter and simultaneously activate several pathways that either positively or negatively regulate cell death induction. This review explains the different processes and pathways that can be targeted to strategize a treatment against head and neck squamous cell carcinoma.*
Keywords: *molecular pathways, strategies, head and neck cancer, nanotechnology, apoptosis*

I. Introduction

Cancer is the second most common cause of morbidity and mortality in the world today after cardiovascular problems. Oral cancer is a significant component of the global burden of cancer; it is the fifth most common cancer reported globally with an annual incidence of over 300,000 cases, of which 62% arise in developing countries. [1]

Though diagnostic techniques have improved over the ages, yet the survival rates remain to be poor and have been the same in the last few years, with a 5-year survival rate of only 50% cases in oral squamous cell carcinoma (OSCC) from the time of diagnosis. [2] The disease shall progress at its own pace and continue to pose as a threat. So, we need to think beyond our present domains and make efforts in the regard.

Although, the recent era has witnessed an exponential growth in the field of cancer; yet the veil of cancer has never been unravelled to detail and its intricacies continue to pose a mystery. Today with the advancements in science and technology attempts have been made to tread deeper into the field of cancer and to evolve techniques and strategies to tackle its ever rising threat.

Oral carcinogenesis has been proposed to be a multi-factorial process that involves various genetic and epigenetic events affecting the normal biology of cells. Normally, a fine balance exists between the proliferation of cells and its programmed cell death. The development of cancer takes place when this balance is disrupted and there occurs uncontrolled proliferation.[3] The transition of normal epithelium to invasive cancer is progressive and accompanied by "multiple hits" or with the up and down regulation of proliferation, angiogenesis, local invasion and, eventually, distant metastasis. [4]

The therapeutic decisions in SCCs are based on clinical and pathological parameters, including age, metastasis stage and histological grade of the tumour. A wider perspective and an insight have to be developed in order to advocate efficacious therapeutic strategies against cancer.

II. Cancer Genetics And Epigenetics

Cancer does not emanate as a modus operandi; it is a methodical course or lineage, its onset may be spontaneous owing to mutations or through a sequential accrue of genetic and epigenetic alterations and the subsequent clonal expansion of the involved cells. [5]

Cancer genomes are predicted to be highly unstable presenting extensive genomic changes ranging from intragenic mutations, to gains and losses of chromosomal material (aneuploidy). Also, epigenomic changes highlight major disruptions in DNA methylation profiles like hypermethylation of gene promoters, global hypomethylation, and also increased rates of mutation at the methylated CpG dinucleotides.

Genomic instability can also be studied; as microsatellite instability (MIN), and chromosomal instability (CIN). MIN refers to a simple DNA base change owing to defects in the DNA repair processes like

nucleotide excision repair, base excision repair and mismatch repair whereas CIN is characterized by its abnormal karyotypes accrued.[6]

As discerned by Hanahan and Weinberg, cells pass through several phases in its biological transition from a normal cell to a neoplastic entity. These sequential stops in progression are summed up as the hallmarks of cancer which also provides for an organizing principle that rationalizes the complexities of neoplastic disease. These are enumerated as sustained proliferative signalling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. [5]

In cancer cells, there is loss of gene expression via underlying genetic and epigenetic mechanisms that target susceptible genes like cell cycle regulators (P16INK4a, P15INK4a, RB, P14ARF), DNA repair genes (BRCA1, MGMT, MLH1), genes associated with apoptosis (DAPK, TMS1), hormonal regulation (ER) detoxification (GSTP1), metastasis (E-cadherin, CD-44), angiogenesis (TSP-1, TIMP-3) and many others. [6]

Tumour progression in oral squamous cell carcinoma evolves through stages of dysplasia (9p21, 3p21,17p13), carcinoma *in situ* (11q13, 13q21, 14q31) and invasive tumours (4q26-28, 6p, 8p, 8q) [10]. According to a recent study on dysplastic lesions of oral mucosa it was noted that there is loss of chromosomal region 9p21 in about 70-80% of the cases along with the inactivation of the remaining alleles of *p16* and *14*ARF via promoter hypermethylation. [7,8]

III. De-Regulated Molecular Signalling Machinery

The cell machinery is a complex circuit intrigued with various pathways, that are involved in signalling and transduction processes in a cell.

So as per the hierarchy in hallmarks of cancer and the most fundamental trait of cancer cells involves their ability to sustain chronic proliferation. Generally the normal tissues lay within the confines and as per the growth-promoting signalling machinery that instruct its progression and homeostasis. Thus, maintaining normal tissue architecture and function. However cancer cells, deregulate these signals by either attaining self-sufficiency/ over-expression of growth signals and their receptors or via insensitivity to growth-inhibitory signals or combination.

1. Mutation Or Over-Expression Of Growth Factor And Receptors

According to many recent studies on cancer, there have been evidence of increased growth factors and their corresponding receptors and also those of receptor tyrosine kinases. These have been attributed to the autocrine and paracrine stimulatory mechanisms alongwith simultaneous coding sequence mutations.

The most widely studied is the epidermal growth factor receptor (EGFR) family (also called HER2/neu and ERBB2) which has been linked to several cancers. [6]

Several mechanisms have been proposed for the receptor's aberrant activation in cancer, which include receptor over-expression, mutation, ligand-dependent receptor dimerization, and ligand-independent activation.The over expression of EGFR is attributed to gene amplification which is noted to be about 12 copies per cell in relation to head and neck squamous cell carcinomas. The constitutive EGFR activation caused via autocrine stimulation and through the co-expression of EGFR with its ligands, TGFα, has been observed in and is indicative of its poor prognosis. [9]

Activated EGFR triggers a lot of downstream signalling events, such as those of Ras/Raf/mitogen activated protein kinase (MAPK) signalling route, the transcription factor signal transducer and activator transcription (STAT), and the phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway, which in turn contribute to the malignant growth and metastatic potential of cancer. [6] EGFR variant III (EGFRvIII) is a truncated mutant form that has proven to be resistant to EGFR blocking antibodies and cisplatin. [10]

The currently approved and under study anti-EGFR agents approved by FDA for treatment of HNSCC are the Monoclonal antibody EGFR-directed therapeutic agents of IMC-C225 Cetuximab (approved) and mAb 2F8 Zalutumumab which is in phase III trial. The Small molecule tyrosine kinase inhibitor EGFR-directed therapeutic agents ZD1839 Gefitinib, OSI-774 Erlotinib are in Phase II – III and GW572016/GW2016 Lapatinib Phase III under trial. [11]

2. Ras–Raf–Mek–Erk Mapk Signalling

Ras are a family of related proteins that are ubiquitously expressed in all cells. On activation by signals the ras proteins regulate proteins involved in cell growth, differentiation and survival. Thus, mutations in ras genes produce an over active ras protein which deranges the signalling. There are 3 ras genes in humans (H-*ras*, K-*ras,* N-*ras*) that are the most common oncogenes in human cancer with an attributable incidence of 20-90%. [6]

Recent studies have highlighted relative high incidence of ras mutations in relation to oral cancer owing to areca nut chewing in Asian populations. Thus, activation of ras in oral cancer may be associated with

either long term exposure to carcinogens or via stimulation of EGFR and growth factor receptors or spontaneous functional mutation of ras gene as in areca nut chewing. [6]

The cascade comprises of three protein kinases that act as a signalling relay. The terminal serine/threonine kinases (MAPKs) are the ERK1/2, the c-Jun amino-terminal kinases (JNK12/3; also called SAPKs), p38 kinases and ERK5. Also studies reveal that the antisense suppression of KSR1 expression was found to impair the growth of ras mutation-positive human tumour cells, supporting KSR as a possible therapeutic target for blocking ras. [11]

The inhibition of ras as a therapeutic development has thus been to block farnesyl transferase, using farnesyl transferase inhibitors (FTIs). The FTIs were under phase III clinical trials R115777/ Zarnestra/ tipifarnib and SCH66336/ Sarasar/ lonafarnib but weren't efficacious cause of prenylation. Thus, an alternative was established involving combination of statins with FTIs. Another recent development is to regulate the expression of ras through the noncoding regulatory RNAs called microRNAs (miRs) that are under trials. Further, the recent FDA approved mitogen-activated protein kinase (MAPK) kinase (MEK) inhibitor is trametinib, trial phase. Also, under research is MEK162 for mutated N-Ras. [6,11]

3. SRC Kinase

Another emerging target is the non-receptor kinase, src, a downstream component of the EGFR signalling complex. Activated src phosphorylates the intracellular domain of EGFR and mediates the activation of the STAT3 pathway by EGFR. Src-kinase is integral to GRP-induced EGFR activation and downstream signalling via the MAPK pathway. In a study, targetting inhibition of src-family tyrosine kinase activity resulted in decreased GRP-induced EGFR activation and MAPK phosphorylation.

Constitutive activation of src may mediate resistance to EGFR inhibitors, and resistance might be overcome clinically by combined inhibition of targets, src and EGFR. Among small molecule tyrosine kinase inhibitors (TKIs) in clinical development, dasatinib inhibits the src family kinase activity in a highly selective manner. [12]

4. Stat Pathway

Signal transducer and activator of transcription (STAT) family consists of 7 members namely STATs 1, 2, 3, 4, 5a, 5b and 6, which coordinate the transcription of genes involved in immune responses, growth, and cell fate decisions. The deregulation of the STAT pathway can render cellular transformational changes and induce oncogenic potential. STAT proteins are stimulated via cytokines and growth promoter factors that lead to recruitment and phosphorylation of Janus kinase 1 and 2 (JAK-1 and JAK-2) that in turn phosphorylate STAT proteins at specific tyrosine residues, thus promoting their homo- and hetero-dimerization. Hereafter, STAT dimers are translocated to nucleus wherein binding to consensus DNA sequences occurs and in turn activates the growth promoting genes(c-myc and cyclin D). [13]

The increased STAT3 levels have been established to deregulate cell cycle progression, promote the proliferation and survival of tumour cells. Head and neck squamous cell carcinoma is associated with elevated levels of the phosphorylated STAT3 and its expression. Also associated is lymph node metastasis and poor prognosis. Studies highlight the activity of STAT5A and STAT5B as over-expressed and phosphorylated, STAT5A causes up-regulation of cyclin D1 and STAT5B inhibition arrests tumour growth.[14,15]Activation of STATs is either direct via EGFR or EGFR-independent mechanisms like autocrine activation of gp130 cytokine receptors via cytokines and erythropoietin. [16,17]

A new line of signal transduction tumour suppressor genes have come to foray, suppressors of cytokine signalling (SOCS) family of STAT-inhibitory proteins, SOCS-1 and SOCS-3, have been efficacious in down-regulating via promoter hypermethylation. Also, some therapeutics strategies target Jak1/2 like INCB018424, which is in phase III clinical trials and it lowers the circulating pro-inflammatory cytokines. Some of the other under study Jak inhibitors are CEP-701, XL019, TG101348 (under trial). [6,12]

5. WNT Pathway

The WNT protein family comprises of 19 cysteine-rich glycoproteins that are known to be an initiating factor for several major signalling pathways, canonical WNT/ β-catenin pathway and non-canonical WNT pathways, c-Jun amino-terminal kinase (JNK), Rho, and calcium signalling pathways. An altered course or parts of the WNT pathway have been observed in oral cancers. Such as studies reveal the increased expression of WNT receptors, Frizzleds, and their downstream target, Dishevelled as contrasted to normal tissue counterparts; studied using gene array analysis, and elevated levels of Wnt14 were noted by mass spectrometric analysis of micro-dissected HNSCC cells. The expressions of natural Wnt antagonists have been reported to be reduced owing to an epigenetic event. [6]

The expression of β-catenin is witnessed to be altered but no supportive mutations have been reported. The hypermethylated APC gene has reduced expression levels along with loss of heterozygosity (LOH) lead to

the altered functioning of the APC tumour suppressor proteins which play a role for the integrity and functioning of the β-catenin destruction complex.[6,19]

Two classes of small molecules inhibiting WNT signalling have been identified. The first is a membrane bound acyl-transferase that inhibits the activity of Porcupine, a molecule essential for WNT synthesis. The second inhibits the destruction of axin, a suppressor of WNT signalling activity. Studies reporting the small molecule iCG001 (institute for Chemical Genomics), to selectively inhibit WNT/ β-catenin signalling by interrupting βcatenin binding to the transcriptional cofactor cAMP response element binding protein (CBP).[18,19]

The use of anti-Wnt-1 antibodies has been advocated against secreted Wnt-1 protein as it inhibits the proliferation and induces apoptosis in cancer cell lines and also relates to a reduced activity of associated transcription factor LEF/TCF activity, and the consequent reduction in cyclin D1 protein expression. Thus, suggesting that WNT presents as a potential target for immunotherapy strategies. [6]

6.   TGF- β Pathway

The transforming growth factor-β (TGF-β) is a part of the superfamily of growth factors that comprises of more than 35 secreted polypeptides. TGF-β acts as a potent tumour suppressor and inhibitor of cell proliferation in many epithelial cells. Secreted in latent forms TGF-βs (L-TGF-β) are activated in acidic environments by proteolysis of matrix metallo-proteinases and plasmins via induced cleavage or conformational changes. They subsequently bind to their receptors (TGFβ RI, TGFβ RII, and TGFβ RIII) and initiate intracellular signalling via Smad and mitogen- activated protein kinase (MAPK) pathways. [20]

TGF-β presents to have a dualistic role both as a potent tumour suppressor during the early stages of carcinogenesis but in its later stages as an adjunct to promoter of tumour progression by facilitating metastatic phenotype. [20]

Approaches established to inhibit the TGF-β signalling pathway are: (1) the inhibition at the translational level using antisense oligonucleotides that can be engineered into immune cells or delivered directly into tumours, (2) inhibition of the ligand-receptor interaction using monoclonal antibodies, and (3) inhibition of the receptor-mediated signalling cascade using inhibitors of TGF-β receptor kinases. Several drugs are either in non-clinical or in early stages of clinical investigation. [21,22]

7.   PI3K / PTEN / Akt/ mTOR Pathway

According to recent studies the phosphatidyl inositol-3-kinase (PI3K) pathway is affected by mutations in its components which accounts for about 30% of all human cancers. The genomic aberrations seen are mutation, amplification, and rearrangements in components of the PI3K signalling route, causing disruption in cellular growth control and survival. [22,23]

According to substrate preference and sequence homology PI3Ks are classified into three classes (I-III). The class IA is activated by EGFR and class IB by GPCRs. Class IA PI3Ks are heterodimers of a p85 and p110 subunits of which p85 binds and integrates signals from various cellular proteins and oncogenic proteins like mutated Ras and Src. [6]

Mutations in PIK3CA gene encoding for p110α, are E542K, E545K and H1047R 41 and other novel mutation (Y343C) within exon 4 have been witnessed in HNSCC (11–40% cases). Also, 3q26 copy number gain has been reported as a common and early oncogenic event in about 40–50% of HNSCC, which has been correlated with transition to a more invasive phenotype, vascular invasion, and a higher probability of lymph node metastasis. [25]

The well studied first-generation PI3K inhibitors in pre-clinical studies are wortmannin (natural), and LY294002 (synthetic drug-like small molecule inhibitor). The wortmannin is known to irreversibly inhibit PI3K whereas LY294002 is an ATP-competitive PI3K inhibitor. However, a relatively advanced novel Arg-GIy-Asp-Ser-(RGDS)-conjugated LY294002 prodrug, named SF1126 and wortmannin analog PX-866 have entered phase I clinical trials. [25]

Reports have indicated a decreased PTEN function in HNSCC due to several genetic and epigenetic alterations. Recently, promoter hypermethylation has been implicated in the down-regulation of PTEN in HNSCC cell lines. These studies were carried out using 5-aza-2'-deoxycytidine (a known demethylating agent) to restore protein expression in cell lines lacking PTEN. [25]

Inhibitor of PDK1 is UCN-01 (7-hydroxystaurosporine) exhibited potent anti-tumour effects in both in vitro and in vivo tumour models. Studies have shown that UCN-01 inhibits the AKT pathway in HNSCC cell lines and the growth of HNSCC xenografts. Several phase I and II clinical trials have already investigated effects of UCN-01 in advanced cancer patients, both as single agent and combined with conventional chemotherapeutic agents. Another potent drug, inhibiting PDK1, is topotecan (10-hydroxy-9-dimethylaminomethyl-(S)-camptothecin), a novel topoisomerase I inhibitor. Also, Irinotecan, a topoisomerase I inhibitor demonstrated single agent activity in patients with metastatic or recurrent HNSCC. [25,26]

The activation status of Akt correlates well with disease progression, showing significant differences between dysplasia, carcinoma in situ and HNSCC tissue. An Akt inhibitor identified perifosine is in Phase II study. [25,26]

mTOR pathway plays a central role in HNSCC, as the phosphorylated active form of S6K1 is frequently accumulated in clinical specimens from HNSCC patients and in HNSCC-derived cell lines. The first mTOR inhibitor was Rapamycin. Recently, rapamycin analogues (rapalogues) have been developed namely temsirolimus (CCI-779), everolimus (RAD001, Novartis) and deforolimus (AP23573).

PI-103, a selective dual PI3K/ mTOR inhibitor showed anti-tumour activity in a broad range of human tumour xenografts, however, application of PI-103 was restricted to preclinical studies because of adverse pharmacological characteristics. Other dual PI3K/ mTOR inhibitors including BEZ235 and BGT226, with promising results from pre-clinical studies, have recently entered clinical trials and may prove useful towards treatment of HNSCC. [25,26]

IV. Loss Of Tumour Suppressor Function
The role of p16 has been pivotal in cell cycle regulation as it binds and inactivates cyclin-bound cyclin-dependent kinases (CDKs) i.e. CDK4 and CDK6 complex, and renders the retinoblastoma protein inactive which further lead to cell cycle arrest. Generally, it's been studied that the pRb, active form is hypophosphorylated wherein it forms a complex with E2F (transcription factor), inhibiting cell cycle progression. On phosphorylation with a mitogen pRb becomes inactive via CDKs, like that of CDK4/ Cyclin D, CDK6/ Cyclin D and CDK2/ Cyclin E complexes. Hence, synthesis of DNA is initiated and orchestrates to endless cell cycle progression. [6,27]

V. Limitless Replicative Potential
A cancer cell is characterized by its tendency to replicate endlessly without any check. The limitless replicative potential of the cancer cell is attributed to interplay of genetic and epigenetic alterations or mutations occurring simultaneously in several genes like that of p16, p53 and the telomerase. The role of p16 has been proved momentous as the silencing of it has been studied in detail of which the major ones being; homozygous deletion, methylation of the promoter, and point mutation. The inactivation of p16 occurs via genetic or epigenetic changes and mutation of p53 that renders the cell to escape cell-induced senescence. The increased telomerase activity on the other hand prevents shortening of the telomeres which subsequently generates signals that infringe on p53 and other related molecules and hamper DNA-damage response. [6,7,12]

VI. Cell Death Pathways
The regulatory mechanisms of cell death are namely necrosis and apoptosis. Cells that are damaged by external injury undergo necrosis, while cells with damaged DNA are induced to commit programmed cell death or apoptosis. Apoptosis involves a complex and sequential signalling module i.e. characterised by two main pathways; the extrinsic or cytoplasmic pathway, triggered via Fas death receptor and the intrinsic or mitochondrial pathway via cytochrome-c. However, both pathways converge to a final common pathway that involves the caspases cascade that ultimately cleaves regulatory and structural molecules leading to cell termination.

Several agents implicate the inhibition of apoptosis related proteins either directly or indirectly. The TNF, Fas, and Fas L are known to have extensive in vitro anti-tumour activity and thus present as potential therapeutic targets in vivo. Agents targeting the extrinsic pathway are the recombinant human TRAIL, monoclonal antibodies (HGS-ETR1, HGSETR2, and HGS-TR2J) agonistic to Dr4 and Dr5, and all trans retinoic acid (ATRA).

Agents targeting the intrinsic pathwayact directly on the mitochondrial inner membrane, or antagonize the anti-apoptotic members of the Bcl-2 protein family, and agents that enhance the activity of the pro-apoptotic members of the Bcl-2 family of proteins such as Bax. The mitochondrial inner membrane agent Arsenic Trioxide targets the PML-RAR protein and is under Phase II trial. Lonidamine, acts on the mitochondria to induce apoptosis through the disruption of the intrinsic transmembrane potential. Agents targeting over-expressed bcl-2 gene is Oblimersen Sodium (G3,139 , Genasense). Few agents involved in modulating the pro-apoptotic proteins bax and bcl-xs are being investigated. The common pathway is marked by a characteristic caspases role; synthetic activators are Apoptin, and IAP targets such as survivin.[28]

VII. Targeting Angiogenesis
The progression of a tumour involves growth, invasion, and metastasis, for which angiogenesis i.e. formation of new blood vessels plays a critical role. Various factors are known to regulate angiogenesis like vascular endothelial cell growth factor (VEGF) has potent angiogenic effects. The presence of VEGF has been

reported in approximately 40% of head and neck squamous cell carcinomas (HNSCCs), and its presence is associated with a poor prognosis. [29]

Recent studies on antisense VEGF mRNA have shown the down-regulation of VEGF and also decreased endothelial migration. To suppress angiogenesis and tumour growth a tumour vaccine targeting VEGF is under animal study. A humanized monoclonal antibody (MAb) inhibiting VEGF, bevacizumab (Avastin) is in phase III study. Also, synergistic study involving bevacizumab, 5-fluorouracil (5-FU) and hydroxyurea with concomitant radiotherapy is in a phase I study of for poor-prognosis head and neck cancer.Investigationsare under way to evaluate the efficacy of dual EGFR-VEGF inhibitors, with preclinical trials using head and neck xenografts demonstrating excellent responses. Another promising inhibitor of angiogenesis, cilengitide, is being currently evaluated in a HNSCC randomized phase II study. [29,30]

Basic fibroblast growth factor (bFGF), platelet-derived endothelial cell growth factor (PD-ECGF), and interleukin-8 (IL-8) are also potent angiogenic factors. The up-regulation of these angiogenic factors in HNSCC makes them ideal targets for molecular therapy. [30,31]

VIII.   Targeting Invasion & Metastasis

With sequential tumourigenesis, the cancer cells acquire the ability for tumour invasion and metastasis. This involves the attachment of tumour cells to the basement membrane, proteolysis of the extracellular matrix and the migration of tumour cells. The adherence is mediated via integrins, E-cadherin, and catenins in head and neck squamous cell carcinoma (HNSCC) tumour cells to the basement membrane. Matrix metallo-proteinases (MMPs) are known to degrade the extracellular matrix and are reported to be up-regulated in 50% of HNSCC cell lines. The targeting of adherence proteins and MMPs may be breakthroughs for molecular therapy. [32]

Urokinase-type plasminogen activator (uPA) and its receptor (uPAR) are up-regulated in HNSCC and are believed to promote tumour invasion and metastasis. The use of anti-uPA antibodies has been shown to prevent tumour invasion in HNSCC cell lines. Likewise, blocking uPAR with molecular inhibitors has been shown to prevent tumour invasion in HNSCC cell lines.

Epithelial cell adhesion molecule (EpCAM) is a transmembrane glycoprotein demonstrated to be up-regulated in HNSCC. Targeted therapies to inhibit EpCAM via usage of monoclonal antibodies such as edrecolomab have had limited success. VB4-845 is currently being evaluated in 2 phase II clinical trials. This drug is a recombinant fusion protein produced by *E coli*, expressing humanized single-chain antibody fragment specific for EpCAM and linked to a truncated *Pseudomonas* exotoxin A. Other strategies such as use of RNA interference techniques to inhibit expression of EpCAM are promising in their pre-clinical trials. [33,34]

IX.   Other Paradigms

Tumour Associated Antigens (TAA)

Molecules that are processed in the intracellular compartment and presented at the cell surface to induce a tumour-specific immune response (immunogenicity) are referred to as timor associated antigens (TAAs). It plays a role in directing immunotherapy and vaccinations regimens. The level of immunogenicity forms the characteristic of tumour specificity and weak target may not be of viable importance.

Some of the TAAs expressed in HNSCC are mutant p53, melanoma-associated antigens (MAGEs), cyclin B1, caspase-8, SART-1, carcino-embryonal antigen, and extracellular matrix metalloproteinase inducer (EMMPRIN) (CD147). [36]

Immunotherapy & Virotherapy

Immune therapeutic approaches are categorised as active or passive and specific or unspecific. Active immune therapy induces an immune response in the host, whereas passive immune therapy is based on the transfer of potent ex vivo pre-treated immune cells or immune globulins (antibodies). For both approaches, the expression 'specificity' refers to the target structure TAAs. Hence, peritumoural injection of a cytokine is defined as passive-unspecific, and TAA-targeted vaccination is defined as active specific. Consequently, infusion of a monoclonal TAA antibody is a passive-specific approach.

The tumourigenesis can also be triggered via HPV infection. Preclinical studies confirmed that HPV-positive tumours induce a specific anti-tumour immune response, which is associated with a better prognosis of HPV-positive HNSCC patients. In cases of patients with HPV-positive carcinogenesis and without any another carcinogenic aetiology is likely to be suitable for any form of HPV-specific immune therapy or virotherapy.[36]

Cancer Vaccines

The tumour vaccination concept suggests that the induction of a tumour-specific immune response in relation to tumour associated antigens (TAAs) on the surface of antigen presenting cells (APCs). The application of TAA-based vaccination may be promising, since it induces an active and specific immune response mediated by cytotoxic T cells or T helper cells. The immune system of HNSCC patients is suppressed

on various levels. The tumour vaccination approach is based on ex vivo generation and cultivation of APCs and consequent loading with TAAs. TAA-specific immune responses are regularly seen after reinfusion of APCs. However, so far these responses were not able to induce a permanent cure for HNSCC patients.

Mutant p53 transcripts are formed due to delayed degradation and are often associated with a strong over-expression of its structurally altered protein in about 80% of HNSCC. The accumulated p53 represents an interesting target structure for the development of tumour vaccines. Efforts have been made to develop p53-specific vaccination strategies, of which the two best known wild-type epitopes are p53149_157 and p53264_272, and other epitopes, histocompatibility leukocyte antigen (HLA)-A2 specific p5365_73 or HLA-A1 specific p53110_124, are also considered as potential ones. The first p53-specific clinical vaccination trial in HNSCC has been initiated and results imply a positive clinical response in the vaccinated patients.

Several melanoma-associated antigens (MAGEs) have been reported to be over-expressed in HNSCC cells. The clinical trials for MAGE-3 have been initiated in HNSCC. Another cancer testis antigen NY-ESO-1 has been identified for specific vaccination in cancer of the oesophagus is under trial. The success of its trial may also provide for a similar regimen for HNSCC. [36]

Gene Therapy

Gene therapy involves the use of DNA as a drug to treat disease by delivering therapeutic DNA into a patient's cells. The most common form of gene therapy involves using DNA that encodes a functional, therapeutic gene to replace a mutated gene.

The reintroduction of wild type p53 in the cells is in its Phase I p53-based gene therapy based trials have shown effective induction of apoptosis in tumour cells and in surrounding cells. The gene therapy technology runs through the obstacle of the requirement to efficiently infect all, or nearly all, cancer cells. In addition, in some animal studies, untransformed thymocytes undergo apoptosis in response to p53 induction, which leads to the need for targeted delivery of transgenes. A large number of adenoviral agents are being developed, including replication-incompetent (eg, rad.p53, SCH5, 8500) and replication selective (ONYX-015; CG7, 870) oncolytic adenoviruses. The agents are introduced by intravascular infusion or intra-tumoural or epi-tumoural injections. Phase I and II studies are under trials.[31,36]

ONYX-015 is a replication-competent virus genetically engineered to selectively replicate in and lyse p53-deficient cancer cells. ONYX-015 has no effect on normal cells. It is given as intra-tumoural and peri-tumoural injections. Phase I and II trials have been conducted in advanced head and neck cancer. However, the development of this compound is currently suspended. [31,36]

INGN 201 is an incompetent adenovirus that delivers a p53 expression. Preclinical studies in human cell lines and animals with head and neck cancers have shown that the p53 gene is efficiently transcribed and translated into p53 protein. Several Phase I and II clinical trials have been conducted. [31,36]

Telomerase Therapy

A cancer cell attains limitless replicative potential or immortality owing to alterations in telomerase end. Strategies are now being proposed that a drug that inactivates telomerase might be effective against a broad spectrum of malignancies. Several studies are being carried out to test the telomerase inhibiting abilities in Inositol hexaphosphate. Also, clinical trials of GRNVAC1 (vaccine) and GRN163L (lapidated oligonucleotide) are being conducted.

Nanotechnology

Nanotechnology is an interdisciplinary research field that involves the expertise of chemistry, engineering, biology and medicine. It has a great potential to aid in early detection, accurate diagnosis and also for personalized treatment of cancer. Nanoparticles, are designed such that they are typically smaller than hundred nanometers in size and may be comparable to large biological molecules like those of enzymes, receptors, and antibodies. Owing to the size of nanoparticles; it offers unprecedented interactions with biomolecules both on the surface of and inside the cells, which may revolutionize cancer diagnosis and treatment. The well-studied nanoparticles include quantum dots, carbon nanotubes, paramagnetic nanoparticles, liposomes, gold nanoparticles and many others. [37]
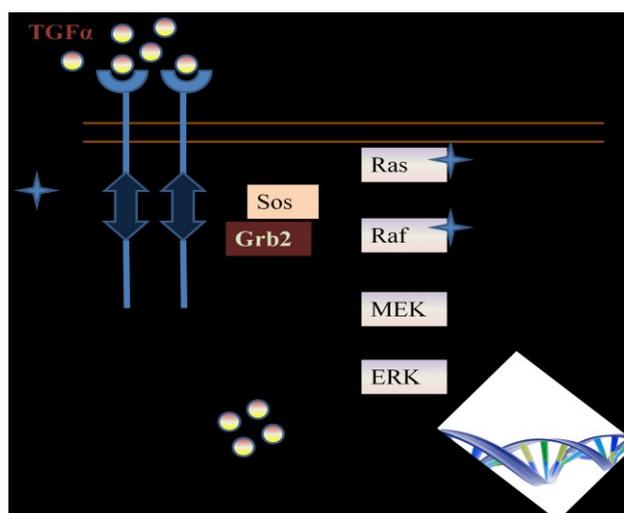
Tables & Figures

Fig 1 :Ras-Raf-MEK-ERK Pathway

Table 1 : Enlisting The Strategies, Targets And The Drugs

Sr. No.	Pathway Targeted		Strategies & Drugs
1	EGFR (epidermal frowth factor receptor)	Monoclonal antibody EGFR-directed therapeutic agents	1. Cetuximab (IMC-C225): Approved 2. Zalutumumab (HuMax-EGFr) (mAb 2F8): Phase I / II trial 3. Panitumumab : Phase III trial 4. Nimotuzumab : Phase II trial
		Small molecule tyrosine kinase inhibitor EGFR-directed therapeutic agents	1. Gefitinib (ZD1839): Phase II / III 2. Erlotinib (OSI-774) : Phase II / III 3. Lapatinib (GW572016/GW2016) : Phase III 4. Others : Afatinib, Dacomitinib, Sunitinib, and Vandetanib : Under Evaluation
2	Ras	Farnesyltransferase inhibitors	1. R115777/Zarnestra/tipifarnib : Phase III trial 2. SCH66336/Sarasar/lonafarnib : Phase III trial
		Non-coding regulatory RNAs	mi-RNA (micro-RNA)
3	MAPK (mitogen activated protein kinase)	MEK (MAPK Inhibitors)	1.Trametinib 2.MEK-162
4	SRC kinase		Dasatinib
5	STAT Pathway (Signal transducer and activator of transcription)	Inhibitory proteins, SOCS-1 &SOCS-3	
		Jak1/2	1.INCB018424 : Phase III 2. Others : CEP-701, XL019, TG101348 : Under evaluation
6	WNT		1.iCG001 : Trial Phase
7	PI3K / PTEN/ AKT/ mTOR PATHWAY	PI3K (phosphatidyl inositol -3-kinase)	1.Wortmannin : Phase I trial 2. LY294002 : Phase I trial
		Akt	1. UCN-01 (7-hydroxystaurosporine) : Phase I / II trial 2. Topotecan : Phase I / II trial 3. Irinotecan : Phase I / II trial
		mTOR (mammalian target of rapamycin)	1.Rapamycin 2.Rapamycin analogs (Rapalogues), temsirolimus(CCI-779), everolimus (RAD001, Novartis) and deforolimus (AP23573) : Under evaluation
		Dual PI3K/mTOR	1.BEZ235 : Phase I / II trial 2.BGT226 : Phase I / II trial

Table No. 2 : Agents Active On Apoptotic Proteins In The Extrinsic, Intrinsic And Common Pathways

Sr. No.	Pathway	Agents	Targets	Stage Of Development
1	Extrinsic Pathway	TRAIL (Tumour necrosis factor related apoptosis inducing ligand receptor)	Dr4 and Dr5	Preclinical
		Monoclonal antibodies agonist to Dr4 and Dr5	Dr4 and Dr5	Phase II / III
		ATRA (All trans retinoic acid)		Clinical use
2	Intrinsic pathway	Arsenic trioxide	Direct effect on mitochondria	Clinical use
		G3139	Bcl2	Phase II / III
3	Common Pathway	Caspase activators : Apoptin and Survivin	Caspases	Preclinical & clinical trials

X.   Conclusion
To conclude an in depth understanding of the complex and intricate cellular signalling cascades and networks with regard to HNSCC may aid for propagating effective therapeutic strategies targeting cancer at the molecular level. The path to clench cancer may seem complicated and versed with difficulties but planned systematic and effective approaches pave the way to render definitive treatment protocols.

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