**METABOLIC SIGNATURE OF SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK: CONSEQUENCES OF *TP53* MUTATION AND THERAPEUTIC PERSPECTIVES**

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**ABSTRACT**

There is a pressing need to identify ways of sensitising squamous cell carcinomas of the head and neck (SCCHN) to the effects of current treatments, both from oncological and functional perspectives. Alteration to cellular metabolism is now widely considered a hallmark of the cancer phenotype; presents a potentially attractive therapeutic target in this regard; and as such has received renewed research interest in recent years. However, whilst metabolic disruption may occur to some degree in all tumours, there is undoubtedly heterogeneity and detailed study of individual tumour types is paramount if effective therapeutic strategies targeting metabolism are to be developed and effectively deployed.In this review we outline current understanding of altered tumour metabolism and how these adaptations promote tumorigenesis generally. We relate this specifically to SCCHN by focusing on several recent key studies specific to SCCHN, and by discussing the role *TP53* mutation may play in this metabolic switch, given the fundamental role of this oncogenic event in SCCHN tumorigenesis. Finally, we also offer insight into the potential therapeutic implications this may have in the clinical setting and make recommendations for future study.

**KEY WORDS**

Head and Neck Neoplasms/drug therapy

Head and Neck Neoplasms/radiotherapy

Head and Neck Neoplasms/genetics

Head and Neck Neoplasms/metabolism

Biomarkers, Tumor/metabolism

Glycolysis/physiology

Glycolysis/drug effects

Glycolysis/radiation effects

Reactive Oxygen Species/metabolism

Tumor Suppressor Protein p53/genetics

**INTRODUCTION**

Squamous cell carcinoma of the head and neck (SCCHN) is the sixth most common cancer globally with an estimated incidence of 750,000 cases per year(1). Whilst tobacco and alcohol consumption are the major risk factors(2), human papillomavirus (HPV) has emerged as a cause of a specific subset of SCCHN(3). Despite advances in diagnostic and therapeutic strategies, survival outcomes for HPV-negative SCCHN have not improved significantly over the last 20 years, still accounting for nearly 350,000 deaths annually worldwide(1). Largely, this reflects the failure of currently available treatment regimes, which are typically multimodal comprising some combination of surgery, ionising radiation (IR), and chemotherapy, to induce a response in advanced disease. In contrast, HPV-positive SCCHN is associated with favourable survival outcomes irrespective of the treatment modality employed(4, 5). This, together with the fact that HPV-driven disease tends to affect younger and generally medically fitter patients, who are therefore likely to experience the functional ramifications of their treatment long-term, has led many to propose treatment de-intensification(6). Despite this apparent clinical paradox, there remains a common goal from a translational research perspective: to identify ways of sensitising these tumours to the effects of current treatments, not only to improve efficacy, but also to minimise the substantial toxic effects. Fundamental to this is to elucidate the cellular processes that may determine radio- and chemo-sensitivity to facilitate therapeutic targeting of the key pathways that may impinge on these processes.

Alteration to cellular metabolism is now widely considered to be a hallmark of the cancer phenotype, intrinsic to malignant transformation, and as such presents a potentially attractive therapeutic target(7). However, whilst disruption of metabolic circuitry may occur to some degree in all tumours, there is undoubtedly heterogeneity, which may reflect both tissue-specific effects and the distinct oncogenic events driving tumorigenesis in different tumour types(8). Consequently, detailed study and consideration of the metabolic phenotype of individual cancers is paramount if effective therapeutic strategies targeting metabolism are to be developed and effectively deployed. Of particular relevance to SCCHN is the fundamental role the tumour-suppressor protein p53 plays in regulating metabolic homeostasis, given the importance of loss of wild-type p53 function in SCCHN oncogenesis(9).

The purpose of this review is to outline current understanding of altered tumour metabolism, how these adaptations promote tumorigenesis generally, and to relate this specifically to SCCHN by discussing the role *TP53* mutation may play in this metabolic switch and by focusing on several recent primary studies specific to SCCHN. We also offer insight into the potential therapeutic implications this may have in the clinical setting.

**TUMOUR METABOLISM AND ROLE IN TUMORIGENESIS**

Whilst it has become clear that tumour metabolism is complex and may differ in many ways from that in normal cells (reviewed in(10)), Otto Warburg’s seminal observation that cancer cells have a propensity to “ferment” glucose remains chief among the recognised metabolic perturbations, is certainly the best characterised, and consequently will be the focus of discussion herein.

Most cells in normal tissues catabolise glucose to pyruvate through glycolysis and, provided there is sufficient oxygen tension, convert pyruvate into acetyl-CoA in the mitochondria before completely oxidising most of that acetyl-CoA to carbon dioxide and water in the tricarboxylic acid (TCA) cycle. This produces reduced nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH2), which then fuel oxidative phosphorylation to maximise adenosine triphosphate (ATP) production. In contrast, cancer cells often have a predilection to direct the pyruvate generated by glycolysis away from the mitochondria to produce lactate regardless of whether or not oxygen is present(11). This is also accompanied by an increase in glucose uptake and glycolytic flux, necessitated by the fact that aerobic glycolysis is considerably less efficient in terms of ATP generation than oxidative phosphorylation(11).

The fact that this switch in glucose metabolism is an energetically inefficient process invites scrutiny of the impetus driving cancer cells to engage in such a process. Whilst Warburg originally proposed that this was compensatory for energy deficiency resulting from defective mitochondrial respiration(12), more recent studies have demonstrated that mitochondrial function is frequently unimpaired in cancer cells(13-15). It has also been suggested glycolytic switching is an adaptive response to hypoxic conditions during the early avascular phase of tumour development(11). However, given that certain leukemic cells and bronchogenic tumours, which exist in oxygen rich environments, also exhibit aerobic glycolysis(16-19) suggests that hypoxia may not be a major factor driving the switch to aerobic glycolysis. Accordingly, such traditional views of tumour metabolism as a self-correcting system have largely given way to more contemporary thinking that metabolic re-programming is a dynamic process adopted by cancer cells to fit the requirements of incessant cell proliferation. Indeed, the ever-evolving links between metabolism and critical oncogenic events, such as *TP53* mutation, Myc and Ras overexpression, persistent activation of phosphoinositide 3-kinase (PI3K)–Akt growth signalling, and enhanced mammalian target of rapamycin (mTOR) activity, lend credence to this notion, suggesting metabolic switching is central to malignant transformation rather than a secondary consequence(20).

The selective advantage that glycolytic metabolism may provide likely relates to the concepts of 1) anabolic biosynthesis to enhance production of proper intermediates to allow for cell growth and proliferation, and 2) regulation of cellular redox potential to minimise the damaging effects of reactive oxygen species (ROS)(11, 20). In order to support proliferation a cell must replicate all of its cellular contents to yield two daughter cells, which, for incessantly proliferating cancer cells, requires liberal production of macromolecular precursors that are generated predominantly from high flux of substrate through glycolysis and associated subsidiary pathways(11, 20). Such increased generation of intermediates, in particular nicotinamide adenine dinucleotide phosphate (NADPH), is also crucial for cancer cells to up-regulate their antioxidant systems to quench the increased ROS levels resulting from heightened oxidative substrate flux, giving rise to the enigmatic situation of high ROS production in the presence of high antioxidant levels(20-22). Although advantageous to tumour cells at moderate levels, excessively high ROS levels result in DNA damage(23), activation of protein kinase Cδ prompting senescence(24), and permeabilisation of mitochondria leading to cytochrome c releaseand apoptosis(21).

In support of these concepts, several independent lines of investigation have demonstrated activation of subsidiary glycolytic pathways in cancer cells, including the hexosamine pathway, uridine diphosphate–glucose synthesis, and the pentose phosphate pathway (PPP)(25-27). Increased activity of the PPP, the major source of cellular NADPH, appears particularly significant given the bipartite importance of NADPH in promoting both macromolecular biosynthesis and redox control(28). In further support of the selective advantage conferred by utilisation of such secondary pathways, many cancer cells preferentially express the M2 isoform of pyruvate kinase (PKM2)(29-31) – a relatively inefficient isoform of the enzyme that catalyses the rate-limiting, ATP-generating step of glycolysis – which facilitates shunting of carbon into these pathways. Similarly, *TP53*-induced glycolysis and apoptosis regulator (TIGAR) also limits glycolytic flux, promotes redirection of metabolites, and is consistently overexpressed in cancer cells(32-34). TIGAR acts principally as a fructose-2,6-bisphosphatase, thereby reducing the concentration of intracellular fructose-2,6-bisphosphate, a powerful allosteric activator of the key glycolytic enzyme phosphofructokinase-1 (PFK-1)(35).

***TP53* STATUS AS A DETERMINANT OF METABOLIC RE-PROGRAMMING**

A fundamental oncogenic event that may contribute to metabolic re-programming is loss of wild-type p53 function. This is particularly pertinent to SCCHN given strong selective pressure for abrogation of p53 tumour suppressor activity in SCCHN tumorigenesis: *TP53* is the most commonly mutated gene in SCCHN, harboured in 60-85% of cases(9), and is associated with a more aggressive and treatment resistant disease phenotype(36, 37). Moreover, in HPV-positive SCCHNs, the HPV E6 oncoprotein specifically inactivates wild-type p53 providing an alternative pathway to the classical mutational “knock-out”(38, 39).

In keeping with its tumour suppressor properties, p53 appears to counteract the metabolic alterations typically associated with cancer development, balancing glycolysis and oxidative phosphorylation through multiple mechanisms(20) (figure 1). PFK-1 represents a key regulatory point in glycolysis and, as discussed above, p53 dampens glycolytic flux at this critical point by inducing the transcription of TIGAR. p53 also represses the activity of the glycolytic enzyme phosphoglycerate mutase (PGAM) through increased ubiquitylation and proteosomal degradation(40), and negatively regulates the expression of pyruvate dehydrogenase kinase (PDK), an enzyme which inactivates the pyruvate dehydrogenase complex (PDC), thus promoting pyruvate decarboxylation and TCA cycle flux (41).

In concert with negatively regulating glycolytic enzymatic activity, p53 also curbs glycolysis by down-regulating relevant transporter molecules: the expression of the glucose transporters GLUT-1 and GLUT-4, which are involved in cellular glucose uptake, is transcriptionally repressed by p53(42), while further downstream in glycolysis p53 suppresses monocarboxylate transporter-1 (MCT-1) expression, which prevents lactate efflux (43).

Complementary to its actions to restrict glycolytic flux, p53 is also coupled to the maintenance of mitochondrial integrity and promotion of oxidative phosphorylation. p53 regulates stability of mitochondrial DNA and mitochondrial biomass through induction of genes such as the ribonucleotide reductase subunit p53R2(44-46), and plays a role in repairing or removing compromised mitochondria via induction of mitochondria-eating protein (Mieap)(47). Specific to oxidative phosphorylation, p53 enhances transcription of Parkin, which promotes mitochondrial respiration through upregulation of the PDC(48). p53 also transcriptionally activates apoptosis inducing factor, which is crucial to the maintenance of electron transport chain (ETC) complex I(49), as well as the genes required for assembly of the cytochrome c oxidase (COX) complex (ETC complex IV)(32, 50-52). Moreover, p53 localised to the mitochondria promotes assembly of ATP synthase (the final ETC complex) in a transcription-independent manner(53).

Further to direct regulatory effects, there is also considerable cross-talk between the p53 system and several other key metabolic mediating pathways, resulting in indirect p53 regulation (figure 2). Perhaps of foremost importance, activation of p53 inhibits activity of two master regulators of cellular metabolism, the PI3K/Akt and mTOR pathways, which ultimately results in suppression of glycolysis. In general, p53 exercises such inhibition through upstream transcriptional regulation. p53 fosters transcription of phosphatase and tensin homolog (PTEN), IGF-binding protein-3 (IGFBP3), tuberous sclerosis protein 2 (TSC2), and the β subunit of AMP-activated protein kinase (AMPK), all of which negatively regulate PI3K/Akt and mTOR pathways(54). Furthermore, p53 promotes expression of sestrins 1 and 2, which also serves to stimulate AMPK activity(55, 56). AMPK was also recently identified to inhibit the hypoxia-induced factor-1α (HIF-1α) pathway(57), which in turn negatively regulates aerobic glycolysis given that HIF-1α acts to enhance the transcription of numerous glycolytic transporters and enzymes(58). To this end, p53 has also been implicated in promoting HIF1-α ubiquitylation and degradation(59). Finally, p53 can inhibit the NF-kβ pathway through inhibition of IKKβ, which also results in decreased glucose transporter expression(60).

Although for the most part p53 acts to repress the malignant metabolic phenotype, seemingly contradictory roles have also been described. For instance, the promoters for the glycolytic enzymes PGAM and hexokinase-II contain p53-responsive elements(61, 62). Whilst the precise reasons for these opposing p53 responses remain to be elucidated, it is well recognised that p53 may act in distinct roles in the wide variety of tissues from which cancers develop(63). It is highly likely, therefore, that p53-mediated metabolic regulation is tissue-dependent(64, 65), further emphasising the importance of cancer-specific study of metabolism.

**METABOLISM IN SCCHN**

Until relatively recently metabolic studies in SCCHN were lacking, and for the most part had focused on isolated or limited transporter/enzyme expression, rather than characterising dynamic metabolic flux and presenting a clear picture of the metabolic phenotype. In this section we briefly discuss these studies and review in greater depth the more recent and robust metabolic studies. Current knowledge of metabolism specific to SCCHN is also summarised in figure 3.

GLUT-1 overexpression relative to normal mucosa and/or benign squamous lesions has been observed consistently(66-70), with one study also demonstrating overexpression in early pre-neoplastic lesions(66), while both GLUT-1 and GLUT-3 overexpression has been reported to negatively prognosticate(71). Importantly, GLUT expression, unlike most other tumour markers, has a biochemical and clinically utilised correlate in 18F-FDG-PET. The glucose analogue 18F-FDG is taken up rapidly into tumour cells by the glucose transporters whereupon phosphorylation by hexokinase prevents release from the cell(72). The absence of the 2' hydroxyl group present in normal glucose prevents further glycolysis, and thus 18F-FDG signal primarily reflects capacity for glucose uptake(72). In addition to successful use in diagnosis, staging work-up, monitoring of response to therapy, and post-treatment surveillance, 18F-FDG avidity is a reliable predictor of long-term survival in SCCHN(72). This, together with the above GLUT expression data, suggests that protein levels are indeed linked to a functional tumour phenotype.

Aside from GLUT expression and activity, only a handful of studies have characterised other metabolic markers in SCCHN. There have been two reports of hexokinase overexpression(73, 74), with one study demonstrating that stable knock-down of hexokinase expression resulted in cell cycle arrest, apoptosis, and reduced viability, suggesting that hexokinase overexpression plays an important role in SCCHN tumorigenesis(74). Two studies have also observed enhanced PDK-1 expression and resulting attenuation of PDC activity(75, 76). Interestingly, normoxic stabilisation of HIF-1α by glycolytic metabolites secondary to PDC inhibition was also observed, which given that PDK-1 is a HIF-1α-regulated gene, suggests a feed-forward effect is at play in contributing to metabolism-mediated malignant progression(75). Reports regarding PKM2 have been equivocal. Although one study found PKM2 expression unsuitable for differentiating oral cavity SCC from normal tissues(77), Wong et al observed PKM2 overexpression in oral tongue specimens(78), and a more recent analysis of the cancer genome atlas RNA sequencing and exon array datasets for SCCHN demonstrated PKM2 overexpression, which correlated strongly to poor prognosis, despite an absence of PKM1 to PKM2 isoform switching(79).

As the final by-product of glycolysis, lactate has also been examined as a proxy of glycolytic activity in several SCCHN studies. Brizel et al demonstrated high pre-treatment tumour lactate levels, which correlated with decreased survival outcomes(80), and a recent prospective study following SCCHN patients for up to 15 years also correlated high lactate levels with both reduced overall survival and tumour recurrence post-irradiation(81). Congruous with this, findings from two *in vivo* studies suggest that increased tumour lactate levels confer relative radioresistance, related to the anti-oxidative capacity of lactate(82, 83). Interestingly, although similar results were observed in a comparative study of cryobiopsies of SCCHN and normal mucosa, expression of lactate dehydrogenase (LDH) (catalyst for conversion of pyruvate to lactate) did not correlate with tumour lactate content(84), which suggests that the raised lactate content may indeed be a by-product of increased glycolytic flux rather than the result of any dysregulation of LDH expression. Recent studies employing nuclear magnetic resonance (NMR)-spectroscopy to enable broad-spectrum metabolomic analyses have also utilised lactate levels to infer glycolytic activity. Two such studies, which scrutinised SCCHN cell lines(85) and snap-frozen tumour specimens(86), demonstrated a diversity of metabolic derangements reliably distinct from normal tissue, with heightened lactate levels a consistent feature. In contrast, however, a further NMR-spectroscopy study reported no evidence of elevated lactate in the observed metabolomic profile, albeit in a relatively small cohort of oral cavity SCC specimens(87).

Another line of evidence indicative of a glycolytic phenotype in SCCHN comes from several therapeutic studies, which have demonstrated the glycolytic inhibitor 2-deoxy-D-glucose (2-DG) to exhibit selective cytotoxicity in both *in vitro* and *in vivo* SCCHN models(88-90). Findings, however, have not been universal(91), and recent evidence from more comprehensive metabolic studies has implicated *TP53* status as a determinant of the metabolic phenotype and therapeutic response. In an initial study, analysis of an isogenic pair of cell lines divergent on *TP53* mutational status revealed a possible role for p53 in determining response to glycolytic inhibition, and by extension the metabolic underpinnings(92). A follow-on study from the same research group examined this further using real-time, quantitative extracellular flux analysis and revealed that mutant *TP53* SCCHN cells exhibit a distinct metabolic phenotype to that of wild-type *TP53* cells whereby wild-type *TP53* cells displayed robust spare mitochondrial respiratory capacity, while mutant *TP53* cells displayed markedly reduced respiratory reserve, functioning near maximal capacity under basal conditions(93). Importantly, this was associated with IR response following glycolytic inhibition, which potentiated IR effects in mutant but not wild-type *TP53* cells(93). Recent preliminary findings from our own laboratory also accord with these results. Utilising a similar experimental system, we recapitulated findings of reduced mitochondrial respiratory reserves and associated sensitivity to the potentiating effects of 2-DG on IR specifically in mutant TP53 cells(94). Moreover, by using specific glycolytic stress tests we also demonstrated a loss of glycolytic reserve in these cells. This allowed further characterisation of the metabolic phenotype, which revealed discernible differences between wild-type and mutant *TP53* cells in their relative utilisation of glycolysis and mitochondrial respiration under baseline conditions(94).

Recognising this association *TP53* mutation and glycolytic dependence, together with the role of the HPV E6 oncoprotein in “inactivating” p53 in HPV-positive SCCHN(39), invites scrutiny of metabolism in HPV-driven disease. However, only one study has addressed this issue to date, examining both resected HPV-positive oropharyngeal SCC specimens and an *in-vitro* HPV-positive cell line(95). Perhaps surprisingly, HPV-positive tumours exhibited increased levels of proteins indicative of oxidative phosphorylation and a relatively lower level of extracellular lactate accumulation(95). It may be the case, therefore, that E6-mediated inactivation of p53 is incomplete, leaving sufficient functional wild-type p53 to maintain a balanced and diversified metabolic profile. Again, however, this study represents an isolated and relatively limited analysis of biomarker expression.

Interestingly, evidence from several of the aforementioned therapeutic studies implicates oxidative stress as the mechanism underlying the cytotoxic effects of glycolytic inhibition, consistent with the proposed impetus driving cancer cells to acquire a glycolytic phenotype, and suggestive of involvement of subsidiary metabolic pathways critical to cellular reducing potential, such as the PPP. For instance, glycolytic inhibition has been shown to cause perturbations in surrogates of oxidative stress, including a decreased proportion of reduced glutathione(88), lessened cellular reducing potential(92), and significantly elevated ROS levels(93). Furthermore, simultaneous treatment with the thiol anti-oxidant N-acetyl cysteine reversed these surrogates together with the therapeutic effects of glycolytic inhibition(88, 92, 93). In keeping with this, a recent study examining metabolism in a matched model of radio-resistant and -sensitive SCCHN cell lines showed that in addition to an increased dependence on overall glucose catabolism, radio-resistant cells also exhibited heightened PPP flux, as evidenced by PPP enzyme overexpression and increased NADPH production(25). Intriguingly, levels of TIGAR expression were also discernibly higher in the radio-resistant cell line, which not only lends further support to increased PPP flux, but also indicates that TIGAR may play a role in such metabolic reprogramming(25). Considering the close regulatory relationship between TIGAR and p53 it is curious that information on the *TP53* status of the radio-resistant cell line was lacking. Indeed, the relationship between p53 and TIGAR in SCCHN represents an area that warrants further investigation given the association between *TP53* mutation and acquisition of glycolytic phenotype, and the fact that uncoupling of TIGAR expression from p53 regulation with TIGAR overexpression has been documented in other cancers(32-34). Further evidence in support of heightened PPP flux in SCCHN comes from three studies examining transketolase (another PPP enzyme), all of which have shown transketolase to be overexpressed in SCCHN cell lines and tissues. These studies also suggested, that transketolase overexpression promotes SCCHN tumorigenicity *in vitro* and *in vivo* through a feed-forward effect on glycolysis(96-98).

Notwithstanding the evidence supporting a glycolytic phenotype, one research group has proposed an alternative model of SCCHN metabolism. In a recent biomarker analysis of SCCHN resection specimens, three metabolically and morphologically distinct cell populations were identified: highly proliferative epithelial cancer cells utilising oxidative phosphorylation with high lactate and ketone body uptake; and non-proliferative epithelial tumour cells and stromal cancer-associated fibroblasts both deficient in mitochondria with elevated lactate and ketone body generation(99). Furthermore, in a subset of specimens both non-proliferative compartments were noted to be highly glycolytic and observed to represent a significant proportion of the tumour(99). Based on this analysis, the authors propose a model of metabolic symbiosis whereby the non-proliferative glycolytic stromal and cancer epithelial cells generate catabolites which are released into the tumour microenvironment and subsequently taken up and utilised by the mitochondrial rich cancer cells to drive energy production and proliferation through oxidative phosphorylation(99). Scrutiny of these findings, however, raises questions over the conclusions drawn. MCT-1, the biomarker employed to indicate catabolite influx in support of mitochondrial respiration, is capable of adapting to facilitate both inward and outward lactate flux(43), while LDH-B, which was used as a glycolytic marker, is an isoenzyme of LDH that drives conversion of lactate back to pyruvate, and is thus more indicative of catabolite influx and reverse glycolysis. There was also a conspicuous absence of *TP53* characterisation, which in light of previous findings relating to the influence of *TP53* status on SCCHN metabolism offers a conceivable explanation as to why only a subset of tumours displayed a significant glycolytic compartment. Similar findings of metabolic coupling, however, have been reported by the same research group for other tumour types(100, 101), and clearly further and more robust exploration in SCCHN is required to reconcile these discrepancies and/or reveal underlying mechanistic explanations.

**POTENTIAL THERAPEUTIC TRANSLATION**

In line with the general consensus that metabolism is substantially altered and intricately linked with malignant transformation, over the last decade cancer metabolism has emerged as an attractive potential target for the development of clinically useful anti-neoplastic agents(102). Therapeutic strategies are generally predicated on a discernible therapeutic index of the chosen agent, either in isolation or in combination with other treatment regimes. Considering this, in contrast to traditional cytotoxic agents, which largely rely on the high and/or incessant proliferation rate of cancer cells rather than true tumour specificity, metabolic targeting can exploit the fact that tumour cells become predominantly dependent on a particular metabolic pathway, providing a selective therapeutic gain while sparing most normal cells. Furthermore, in the context of SCCHN, for which IR and cisplatin are the mainstays of non-surgical management(103, 104), targeting the “addiction” to glycolysis in combination with these conventional treatments appears particularly attractive. The anti-tumorigenic effects of both IR and cisplatin are to some extent dependent on intracellular reducing potential: IR induces DNA damage through a mechanism that relies on free radical formation(105), while cisplatin is inactivated by reduced glutathione and other thiol-containing proteins(106). This, taken together with accumulating evidence implicating oxidative stress as the mechanism underlying the therapeutic effects of glycolytic inhibition in SCCHN, provides further support for a therapeutic advantage, preserving normal cells, which do not carry such a high burden of oxidative stress. In addition, recovery following IR and cisplatin-induced damage, requiring activation of DNA damage repair pathways and cisplatin export, is energetically demanding, rendering tumour cells yet more vulnerable to antimetabolic agents.

Accordingly, as touched on above, a number of therapeutic studies have demonstrated that the apparent glucose addiction of SCCHN cells does indeed translate into a selective sensitivity to glycolytic inhibition, particularly when combined with conventional treatments (summarised in table 1)(88-94, 107, 108). The majority of these comprise *in vitro* studies using homotypic cell culture experiments focusing on 2-DG, while one pre-clinical *in vivo* study has shown 2-DG to inhibit growth of SCCHN xenografts both in isolation and in a combined-modality approach with cisplatin(90). Following on from these pre-clinical studies, only two clinical studies to date, both originating in Italy in the mid 1990s, have evaluated the role of metabolic targeting in SCCHN(107, 108). These clinical trials both examined the effects of oral administration of the glycolytic inhibitor lonidamine, in one setting in combination with chemotherapy (methotrexate) in 89 patients with recurrent or metastatic SCCHN in a phase II randomised design(107), and in the other in combination with IR in 96 patients with advanced primary SCCHN as a phase III, double-blind, randomised, placebo-controlled trial(108). Intriguingly, results suggested that the addition of lonidamine in either instance improved clinical outcomes, and was seemingly tolerable, with only minor side effects and relatively low attrition rates reported(107, 108). Specifically, in the phase III study although there was negligible difference in initial disease control rates (66% in the lonidamide group versus 65% in the placebo group), the addition of lonidamine resulted in a lower subsequent treatment-failure rate following initial disease clearance (50% versus 77%), and significantly improved loco-regional control and disease-free survival at three and five years(108).

In view of these findings, it is perhaps surprising that there has been such a dearth of further clinical studies investigating this issue. Considering findings from other solid tumour types, however, highlights that whilst in many instances clinical results have been therapeutically promising, responses have been inconsistent, even when combined with conventional therapies(109). Whilst these discrepancies may simply reflect differing metabolic profiles in different tumour types, and thus variable responses to glycolytic inhibition, another possible explanation relates to a lack of available predictors to define patient populations most likely to derive therapeutic benefit. Indeed, in light of the aforementioned recent *in vitro* findings from SCCHN studies(92-94), which have revealed links between *TP53* status, metabolic phenotype and response to glycolytic inhibition, it is intuitive to suggest that in SCCHN tumours harbouring *TP53* mutations, concurrent glycolytic inhibition could result in significant chemo- and/or radiosensitisation, while in those expressing wild-type p53 a broader antimetabolic approach would likely be required. To this end, it is important to note that both xenografts used in the pre-clinical *in vivo* study mentioned previously originated from cell lines harbouring *TP53* mutations(90). This proposed paradigm represents a unique therapeutic opportunity to preferentially address the typically aggressive and treatment-resistant disease associated with *TP53* mutation(36), and should foster future clinical studies in SCCHN, which would aim to stratify patients on this basis.

Interestingly, combining 2-DG with the PPP inhibitor 6-amino nicotinamide results in an enhanced cytotoxic effect in SCCHN cells(25, 110), which lends further support to the notion that inhibition of detoxifying antioxidant systems has potential therapeutic gain, particularly as a viable means of potentiating the effectiveness of traditional therapies, and further suggests that this may be optimised by combined pharmacological targeting. Whilst 2-DG, the most extensively studied glycolytic inhibitor, competes for glucose uptake and processing by glycolysis, thereby creating a drug-induced state of glucose deprivation, it does not completely inhibit the generation of NADPH through the PPP. 2-deoxy-D-glucose-6-phosphate (phosphorylated product of 2-DG by hexokinase) can be oxidised to 2-deoxygluconate-6-phosphate by the first PPP enzyme, leading to the regeneration of one molecule of NADPH(111), and the products of gluconeogenesis feeding into the PPP may still occur in 2-DG treated cells. Thus, inhibition of glycolysis and PPP by 2- DG alone does not inhibit NADPH regeneration maximally, and distinct PPP inhibition is required to restrict NADPH production and upregulation of cellular anti-oxidant systems. In the absence of clinical studies, however, whether a therapeutic window for the clinical application of PPP inhibitors exists remains to be determined.

It is also important to consider the proposed three-compartment model of tumour metabolism in SCCHN given the potential therapeutic implications. In this model programmed exchange of catabolites between proliferative and non-proliferative compartments is largely driven by MCT-1 and MCT-4, which suggests pharmacological agents should be directed against these transporters to target metabolic symbiosis(99). A potent MCT-1 inhibitor, α-cyano-4-hydroxycinnamate (CHC), has demonstrated anti-tumorigenic effects *in vitro* and *in vivo*(112, 113), while another MCT-1 inhibitor, AZD-3965, is currently undergoing clinical trials in patients with advanced cancers (ClinicalTrials.gov identifier: NCT01791595). Although no agents that selectively disable MCT-4 are currently available, studies utilising functional inhibition with RNAi have demonstrated antineoplastic effects *in-vivo*(114), highlighting the need for development of potent small molecule inhibitors directed against MCT-4.

A major issue remains the skepticism over the proposed therapeutic window for metabolic targeting, which stems from concerns over uniformity between metabolism in cancer cells and that in highly proliferative normal cells or in those primarily dependent on glucose catabolism, such as red blood cells and neurons. Unfortunately, the limited number of clinical studies conducted to date on anti-metabolic agents makes it difficult to predict whether this truly constitutes a liability in therapeutic settings. Nonetheless, many agents that act on metabolic enzymes have been used clinically for decades without detriment, indicating that a therapeutic window may indeed exist for harnessing the anti-tumorigenic effects of these agents in clinical settings(102).

**CONCLUSIONS**

In this review we have discussed the role of altered metabolism in tumorigenesis generally, and more specific to SCCHN we have explored the potential role of p53 in metabolism and the existing data particular to SCCHN. In general, there is convincing evidence that aerobic glycolysis constitutes the metabolic signature of SCCHN, likely with utilisation of subsidiary pathways, such as the PPP. This seems to be particularly so in advanced and treatment resistant disease, and recent evidence has demonstrated that this metabolic switch is driven by mutational loss of wild-type p53 function. Importantly, this appears to make this tumour type susceptible to the potentiating effects of glycolytic inhibitors as radio/chemo-sensitisers, which offers the opportunity for a novel and tailored anti-metabolic therapeutic paradigm for SCCHN, which not only carries a selective therapeutic index, but is also informed by *TP53* status as a predictive biomarker. This strategy would be applicable in upwards of 60-85% of SCCHNs and would be preferentially effective in patients with the aggressive and treatment-resistant disease typically associated with *TP53* mutation.

The majority of experimental evidence to support this, however, comes from *in vitro* homotypic culture studies. As such, we recommend that future work should focus on comprehensive investigation of known antiglycolytic agents in a more relevant pre- clinical setting including organotypic *in vitro* platforms and *in vivo* orthotopic animal models. Such experimental platforms would also model potential complex interactions between tumour, surrounding stroma, and host, which would be of particular interest given the possibility of metabolic symbiosis in SCCHN. In addition, we suggest that future work should examine more comprehensively the role of the PPP and associated therapeutic targeting considering the mechanistic links between metabolic switching and upregulation of cellular antioxidant systems to quench excessive ROS accumulation, together with the role of the PPP in generating reducing equivalents to fuel cellular antioxidant systems. A further line of investigation should also be directed towards characterising the metabolic phenotype and exploring anti-metabolic therapeutic avenues in HPV-positive disease given the paucity of studies investigating this to date. Moreover, any means of radio-/chemo-sensitising these tumours could provide an attractive platform for treatment de-intensification by facilitating RT and/or chemotherapy dose reduction to minimise the impact of treatment on long-term function. Ultimately, we believe that combining these approaches will lead to significant improvements in our understanding of SCCHN tumorigenesis, with the potential for translation into significant clinical advances.

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