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Co-targeting the *EGFR* and *PI3K*/Akt pathway to overcome therapeutic resistance in head and neck squamous cell carcinoma: What about autophagy?

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ABSTRACT. Recent insights in the *PI3K*/Akt pathway as a promising therapeutic target in combination with *EGFR*-targeting agents to treat head and neck squamous cell carcinoma. The Epidermal Growth Factor Receptor (*EGFR*) is overexpressed in the majority of Head and Neck Squamous Cell Carcinomas (HNSCC). This triggered the development of multiple anti-*EGFR* agents as a potential treatment strategy for this disease. Despite initial promising results and clinical implementation of one of the first successfully approved targeted therapies in solid tumor treatment, namely the *EGFR*-specific antibody cetuximab, intrinsic and acquired resistance often occurs with a negative effect on outcome.

Key words: Catalytic; Autophagy; Mutations; Downstream; Effector

INTRODUCTION

The Epidermal Growth Factor Receptor (*EGFR*) is overexpressed in the majority of head and Neck Squamous Cell Carcinomas (HNSCC) [1,2]. This triggered the development of multiple anti-*EGFR* agents as a potential treatment strategy for this disease. Despite initial promising results and clinical implementation of one of the first successfully approved targeted therapies in solid tumor treatment, namely the *EGFR*-specific antibody cetuximab, intrinsic and acquired resistance often occurs with a negative effect on outcome. In theory, pharmacological blockade of *EGFR* should result in the inhibition of its major downstream signaling pathways, i.e., (i) the Ras/Raf/Mitogen-Activated Protein Kinase (MAPK) pathway; and (ii) the phosphatidylinositol 3-kinase (*PI3K*)/Akt pathway. However, it is becoming more and more clear that the *PI3K*/Akt pathway often remains activated in patients who exhibit *EGFR*-targeted therapy resistance [3,4]. In this light, co-targeting *EGFR* and the *PI3K*/Akt pathway might be a promising therapeutic strategy to overcome anti-*EGFR* therapy resistance in HNSC.

LITERATURE REVIEW

Genetic background of resistance to anti-*EGFR* therapies: Focusing on the *PI3K*/Akt pathway

Despite anti-*EGFR* therapy, the sustained activation of the *PI3K*/Akt pathway, might be explained by looking at the genetic background of the resistant tumor. The presence of activating mutations in genes that lead to the overexpression and sustained activation of key mediators of the *PI3K*/Akt pathway might be involved in the development of resistance 1 [5]. Interestingly, the *PI3K*/Akt pathway is one of the most frequently mutated pathways in HNSCC [6]. Genetic alterations in one of the major components of this pathway are seen in 66% of HNSCC patients [7]. On the other hand, as an increasing proportion of HNSCC are Human Papilloma Virus (HPV) positive, it is worth mentioning that HPV infection can also lead to aberrant activation of the *PI3K*/Akt pathway. The HPV oncoproteins E6 and E7 have been shown to activate major components of the pathway [8,9] and might therefore also play a role in *EGFR*-targeted therapy resistance.

According to the TCGA dataset, mutations in the *PIK3CA* gene, which encodes for the catalytic p110 subunit of *PI3K*, can be found in 21% of HNSCC patients and are common in both HPV-positive and HPV-negative HNSCC7. *In vitro* studies have also shown that cell lines that display activating *PIK3CA* mutations were characterized by an inadequate response to cetuximab treatment, suggesting a role of *PIK3CA* mutational status in cetuximab resistance.

Additionally, the *PI3K*/Akt pathway is negatively regulated by the tumor suppressor phosphatase and tensing homolog (PTEN), which dephosphorylates PIP3, thereby terminating the signaling cascade. Loss of this protein results in a release of the internal breaks on the pathway and is often associated with more aggressive tumors. In HNSCC, low or complete loss of PTEN expression is observed in 10%-30% of the patients, regardless of the HPV status [6,7,10-12]. It has been shown that PTEN loss may reduce the effectiveness of multiple *EGFR* inhibitors in HNSCC [13,14]. In addition, there are several indications that it might serve as a predictive biomarker for anti-*EGFR* therapy response [15].

Mutations in genes encoding for the last two major downstream effector molecules of the *PI3K*/Akt pathway (Akt and mTOR) are almost non-existing, whereas significant overexpression of these proteins is more frequent [16]. In this regard, increased levels of phospho-Akt following cetuximab treatment [17] and elevated mTOR activity [18] have been reported in HNSCC resistance studies, although their precise role in anti-*EGFR* resistance remains largely unclear.

Co-targeting *EGFR* and *PI3K*/Akt pathway: Match made in heaven or not?

As mentioned above, HNSCC tumors resistant to *EGFR* inhibitors are often characterized by genetic changes in major players of the *PI3K*/Akt pathway. Therefore, simultaneous targeting of *EGFR* and the *PI3K*/Akt pathway seems to be a logical step in order to overcome anti-*EGFR* therapy resistance. Indeed, multiple preclinical studies have demonstrated promising results, showing that combining *EGFR* and *PI3K* pathway inhibitors often leads to superior anti-tumor effects compared to either treatment alone. Several underlying mechanisms could be responsible for the effect observed in these combination treatments and one of them is the induction of autophagy.

Autophagy is an evolutionary conserved catabolic process in which cells sequestrate, degrade and recycle their own intracellular contents, such as organelles and proteins. It is mainly known as a protective mechanism against cellular stress, with the goal to provide the cell with the necessary nutrients in order to survive. However, autophagy can also be a mechanism of programmed cell death (autophagic cell death, ACD), although there is still controversy over the definition or even existence of ACD [19].

All signaling pathways downstream of *EGFR* are involved in the regulation of autophagy. *EGFR*-mediated Ras/Raf/MAPK signaling promotes the autophagic response through serine phosphorylation of Beclin1, while signaling through the *PI3K*/Akt pathway suppresses autophagy by activating mTOR, which is a well-known inhibitor of autophagy [20]. Therefore, blocking both *EGFR* and the *PI3K*/Akt pathway as a therapeutic strategy to overcome resistance of *EGFR*-targeted agents in HNSCC may result in an increased activation of the autophagic response, leading to either cell survival or cell death. Which path will be chosen (cell survival or cell death) is uncertain and depends on multiple factors, such as cell type, genetic background and prevailing microenvironment? For example, radiation-induced autophagy was shown to be cytoprotective in the p53 wild-type HNSCC HN30 cell line, while it was nonprotective in the p53 mutant HN6 cell line [21]. Unfortunately, it remains challenging to elucidate the specific cellular requirements to promote ACD.

Activation of autophagy during anti-*EGFR* therapy is commonly seen as a bad sign, as there are several indications that autophagy induction might be a treatment escape mechanism and thus involved in the development of therapeutic resistance. In this regard, increased expression of the selective adaptor protein in autophagy p62 has been associated with cetuximab resistance [22]. Activation of autophagy by induction of oxidative stress was shown to diminish the efficacy of erlotinib in HNSCC cell lines [23]. Likewise, *PI3K*/Akt pathway inhibitors have been reported to induce protective autophagy, supporting unwanted cell survival of tumor cells. In this context, combining *PI3K*/Akt inhibitors with autophagy inhibitors, such as hydroxyl chloroquine, has demonstrated superior anti-proliferative effects in HNSCC cell lines compared to *PI3K*/Akt inhibitors alone [24].

The latter studies do not quite advocate for the dual targeting of *EGFR* and *PI3K*/Akt pathway inhibitors as a promising strategy to overcome resistance in HNSCC. However, as mentioned previously, autophagy can also be a mechanism of cell death, although this cytotoxic element remains largely unclear and warrants further investigation. In addition, autophagy plays a role in anti-tumor immunity, as it ensures the release of antigens, potentially leading to tumor recognition and elimination [25]. Therefore, it is worth investigating autophagy as an anti-tumor mechanism in HNSCC. In this regard, the study of D. Amato et al. demonstrated that there was a highly synergistic effect when cetuximab was combined with the dual *PI3K*/mTOR inhibitor PKI-587 in both cetuximab sensitive and resistant cell lines. Interestingly, cetuximab sensitive cell lines were characterized by activation of apoptosis, compared to cetuximab resistant cell lines that showed activation of autophagy. More specifically, resistant cell lines had an increased expression of Beclin1 and a decreased expression of p62 following combination treatment. Since a significant inhibition of cancer cell proliferation was observed in all cell lines, the authors concluded that the combination of PKI-587 and cetuximab induced apoptotic cell death in sensitive cell lines and a different type of cell death, i.e., ACD, in resistant cell lines. Thus, in this study, the observed induction of autophagy was not mediating therapy resistance, as often described in literature [22-24], but rather overcoming it as an indirect effect of the combination regimen.

DISCUSSION AND CONCLUSION

Aberrant signaling of the *PI3K*/Akt pathway is involved in resistance to *EGFR*-targeted therapies. Genomic alterations in and/or overexpression of the major components of the *PI3K*/Akt pathway are common in both HPV-positive and HPV-negative HNSCC tumors. Therefore, downstream effectors of the *PI3K*/Akt pathway serve as promising targets in the search for novel therapeutic strategies that are able to overcome resistance to *EGFR* inhibitors. *EGFR* and *PI3K*/Akt pathway inhibitors or combinations thereof can induce an autophagic response in tumor cells. The induction of autophagy during anti-cancer therapy can be seen as a "double-edge sword", depending on the cellular context. The role of therapy-induced autophagy following dual targeting of *EGFR* and the *PI3K*/Akt pathway as an anti-tumor mechanism is still largely unclear in HNSCC. Further research is warranted to fully understand the potential of combination treatments targeting both the *EGFR* and *PI3K*/Akt pathway.

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