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(PI3K)/AKT signalling pathway – a Pandora's box in oral squamous cell carcinoma

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Abstract

Molecular mechanisms of cancer involve mutations of several genes triggering various pathway alterations. Oral squamous cell carcinoma (OSCC) represents approximately 10% of the head and neck cancers and is one of the leading causes of morbidity and mortality in Central and Eastern Europe. The main aim of this review article is to present an overview of one of the major actors in cellular processes' regulation that are key features of cancer molecular events - the PI3K/AKT signalling pathway. The altered molecular signalling pathways in OSCC have been discussed, emphasizing the frequent disruption of the PI3K/AKT pathway in cancer. Thus, the aspects presented in this article highlight this pathway as an important target for new therapeutic strategies.

Keywords: oral squamous cell carcinoma, PI3K/AKT signalling pathway, p53 protein

INTRODUCTION

Cancer has a multi-step molecular mechanism, within which several mutations accumulate, leading to:

- initiation, progression, maintenance of proliferation
- signalling evasion of growth suppressors
- angiogenesis
- continuous replication
- avoidance of apoptosis
- invasion and metastasis

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Molecular mechanisms of cancer involve mutations of several genes triggering various pathway alterations (1-4).

Among the head and neck cancer cases, approximately 10% are represented by oral squamous cell carcinoma (OSCC) (5,6). OSCC is the most common malignancy of the oral cavity and the sixth most frequently diagnosed cancer worldwide (5, 6). Unfortunately, most OSCC patients are usually diagnosed after the cancer has reached an advanced stage. This is the reason why this type of tumor is generally associated with poor prognosis. OSCC is one of the leading causes of morbidity and mortality in Central and Eastern Europe (1).

Despite the progress regarding cancer diagnosis and treatment, the overall 5-year survival rate for OSCC still remains the lowest among malignancies (2).

OSCC can appear anywhere in the oral cavity, including upper and lower gingiva, the tongue, oral floor, palate, and buccal mucosa. Anatomically, OSCC of the tongue and gingiva is able to invade the adjacent muscles and the jaw bones, respectively. Moreover, OSCC has an accentuated tendency to initiate cervical lymph node metastasis because oral cavity lymphatic vessels are rich and comprise numerous anastomoses (3). Furthermore, OSCC causes serious chewing and swallowing dysfunctions, as well as aesthetic and speech disorders, worsening patients' life quality (4).

The specific molecular prognosticators for OSCC have not been identified yet.

Not long ago, Hanahan and Weinberg (7) proposed "10 hallmarks of cancer", true focal points in cancer characteristic evolution:

- evading growth suppressors
- genome instability and mutation
- disruption of the energetics
- enabling replicative immortality
- resisting cell death
- avoiding immune destruction
- sustaining proliferative signalling
- inducing angiogenesis
- activating invasion and metastasis
- tumor-promoting inflammation.

In order to elaborate new cancer treatment strategies, it is impetuously necessary to identify precisely all the molecular pathways involved in oral cancer progression. Recent studies analysing the oncogenic signalling pathways, suggested important roles on the oral cancer stage for glycogen synthase kinase 3 (GSK-3β), c-Myc, AKT (protein kinase B), β-catenin, p53 protein, and nuclear factor kappa B (NF-kB) (8).

Identifying these molecular signalling pathways is quite important, since their therapeutic inhibition could seriously improve the patient survival rate.

More and more studies are focusing on the phosphatidylinositol 3-kinase (PI3K)/AKT signalling pathway in the context of OSCC. It has been shown that this molecular pathway is quite frequently disrupted in cancer and consequently represents an important target for new therapeutic strategies (9).

The PI3K/AKT signalling pathway is a major actor in regulating cellular processes that are key features of cancer molecular events, such as:

- survival
- cell proliferation
- migration

Consequently, over the last years, more and more molecules targeting PI3K or AKT, key members of the PI3K/AKT signalling pathway, have been extensively developed and have been tested in clinical trials (10). Unfortunately, similarly to what has been noticed for other targeted therapies, adaptive resistance intervened, limiting the antitumor action of these drugs. Thus, the complete elucidation of all possible interactions between the components of the pathway is very important, in order to elaborate new therapeutic concepts that may be exploited clinically.

PI3K AND *AKT*, THE KEY MEMBERS OF *PI3K/AKT* SIGNALLING PATHWAY

PI3K (phosphatidylinositol 3-kinase) represents a family of lipid kinases. It has been pointed out that PI3Ks play key roles in complex cellular functions such as cell proliferation and survival (11, 12).

PI3Ks generate phospholipids meant to transduce the signals generated by G protein-coupled receptors and, especially, by receptor tyrosine kinases. Considering their structure and substrate specificity, PI3Ks are classified into three distinct categories: Class I, II and III (13).

Class I PI3K is composed of Class IA and IB. Among the classes of PI3Km, the IA PI3Ks appear to have the predominant role on the cancer stage.

Structurally, these enzymes are composed of:

- a catalytic p110 subunit responsible for generating phosphatidylinositol 3, 4, 5-triphosphate (PIP3)
- a regulatory p85 subunit, responsible for the PI3K interaction with the upstream effectors (13).

Class IA PI3K enzymes activation occurs mostly through tyrosine kinase receptors (13). Following activation, Class I PI3Ks generate PIP3 at the plasma membrane. The new formed PIP3 will trigger

the recruitment of proteins like PDK1 (pyruvate dehydrogenase lipoamide kinase isozyme 1) and AKT (14).

PIP3 levels are tightly controlled by the activity of the tumor suppressor PTEN (phosphatase and tensin homolog). PTEN converts PIP3 back to phosphatidylinositol 4,5-bisphosphate, thus acting opposite to PI3Ks (15).

The PIP3 triggered recruitment of PDK1 and AKT to the plasma membrane leads to the interaction of both kinases. Consequently, PDK1 will phosphorylate and, thus, will partially activate AKT (16-17). However, complete AKT activation further requires an additional phosphorylation by mTORC2 (18).

Completely activated, AKT will phosphorylate specific downstream effectors, including FOXO (Forkhead box O transcription factor), GSK-3β (glycogen synthase kinase 3 beta), p27, PRAS40 (proline-rich AKT substrate of 40 kDa), BAD (Bcl-2-associated death promoter), TSC2 (tuberous sclerosis complex 2) and Caspase-9. These downstream effectors' phosphorylation will ultimately result in cell growth and proliferation, key events in cancer (19).

There have been described three different AKTs molecular species (AKT1, AKT2 and AKT3), products of three different genes, with 80% amino acids homology (20). AKT1 promotes cell growth and survival, whereas AKT2 controls cellular invasiveness (20,21).

HOW *OSSC* CELLS MANAGE TO AVOID THE GROWTH SUPPRESSORS? *PTEN* AND *AKT* MAIN ACTORS

In cancer cells, many tumor-suppressor genes are inactivated by methylation, deletion, and mutations.

An important ally of normal oral mucosa cell is p53 protein. It has been established that p53 represents a real "genome guardian", playing a key role in cell cycle regulation, apoptosis, cellular differentiation and DNA repair (22).

p53 mutations were detected in 60–80% of OSCC cases and in 10% of oral dysplasia cases (23). Recently, in the context of OSCC, p53 mutations were associated with reduced sensitivity to cisplatin, extranodal extension, distant metastasis, finally leading to poor prognosis (24).

p53-mutant OSCC patients' overall survival is considered markedly worse than that of p53 wild-type patients (25).

Moreover, PTEN plays the role of an important tumor repressor, acting through a negative feedback of the phosphoinositide 3-kinase (PI3K)–AKT pathway (26). Also, PTEN is an insulin signalling inhibitor by indirect suppression of mitogen-activated protein kinase (MAPK) and insulin receptor substrate 1 (IRS-1) phosphorylation (26).

Consequently, in OSCC, PTEN expression inhibition is considered a predictor for unfavorable prognosis (27). PTEN gene expression inhibition may be due to methylation as long as PTEN mRNA restoration has been noticed after a treatment with 5-aza-20-deoxycytidine (5-Aza-dc), a demethylation agent, in human OSCC-derived cells (28).

PI3K AND *AKT* – PASSIVE ENEMIES IN CHALLENGING *OSCC*?

The molecular mechanisms responsible for these experimental findings have not been fully understood yet. These mechanisms might include:

- a PIP3 persistent low level, able to maintain AKT phosphorylation,
- phosphorylation by another kinase than mTORC2 (29).

These experimental findings clearly illustrate that cancer cells, including OSCC cells, are able to adapt to the inhibition of a specific isoform of PI3K by restoring the AKT initiated signalling via another PI3K isoform (30).

PI3K inhibitors effects on AKT activity may, also, vary in time as cancer cells need time in order to restore AKT activity following the exposure to PI3K inhibitors. Consequently, serial tumor biopsies from the PI3K inhibitors treated cancer patients should be required, which is not ethically justified. One key lesson drawn from the results of clinical trials using targeted therapies is that much more attention should be on the emergence of acquired resistance to those agents by cancer cells as well as by the tumor microenvironment (31).

PI3K inhibitors studies revealed more and more clearly resistance mechanisms used by cancer cells against these inhibitors. For example, it has been identified a mutation of the p110α subunit that confer resistance to PI3K inhibitors. Additionally, in breast cancer, IL-8 (interleukin-8) secretion, result ofnJAK2/STAT5 (Janus kinase 2/signal transducer and activator of transcription 5) activation, has also been shown to block the anticancer activity of PI3K inhibitors (29).

At this moment it is quite clear that cancer cells, including OSCC cells are very capable of developing resistance mechanisms to PI3K inhibitors, which should be regarded as extremely important targets in order to design new therapeutic strategies. These new therapeutic strategies should be able to overcome all these molecular resistance mechanisms and improve the anticancer action of PI3K inhibitors.

Moreover, because PI3K inhibitors cannot induce persistent AKT signalling inhibition, a hopeful idea would be the combination of PI3K and AKT inhibitors in cancer therapy.

CONCLUSIONS

So, finally it can be assumed that targeted therapies, like PI3K inhibitors treatment, used alone, are not able to treat patients with advanced OSCC. In order to obtain stronger anticancer effects, it is necessary to design combined therapies.

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