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REVIEW

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DNA damage response and potential biomarkers of radiosensitivity in head and neck cancers: clinical implications

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Abstract

Head and neck cancers include a wide variety of tumor sites that originate in the epithelium of the upper aerodigestive airways. The curative treatment of this group of pathologies most frequently involves multidisciplinary approach in which radiotherapy (RT) plays a central role. Treatment failures are mainly due to recurrences and local or regional evolution and rarely to distant metastases, which emphasizes the importance of ensuring local control. For patients with recurrences, the treatment options are significantly reduced, and prognosis is considerably attenuated. At the cellular level, the main irradiation target is the deoxyribonucleic acid (DNA), its lesions being largely responsible for radiation-induced cell death. However, not all DNA damage will have the same biological significance and a considerable part will be repaired through an intricate network of signaling proteins and repair pathways. Radiobiologically, compared to normal cells, tumor clonogens are defined by malfunction of DNA repair pathways. Tumors with an increased repair capacity, especially DNA double-strand breaks, the most lethal lesions induced by RT, will be radioresistant. The purpose of this review was to elucidate the mechanisms involved in avoiding radiation-induced apoptosis of head and neck cancers mediated by modulating the repair of DNA damage *via* p53, epidermal growth factor receptor (EGFR) and p16. The role of DNA damage-associated biomarkers in response to irradiation in clinical practice for the selection of personalized treatments and specifying the prognosis and, finally, the bases of immunotherapy association are presented.

Keywords: DNA damage, DNA repair, head and neck cancer, radiotherapy, biomarkers.

Introduction

Head and neck cancers include a wide variety of tumor sites that originate in the epithelium of the upper aero-digestive tract. With an incidence of over 900 000 new cases worldwide annually, it ranks 7th among the most common tumor locations [1].

The topographic location influences the spread and prognosis, but at the same time there is a series of common elements in terms of etiology, histogenesis, evolution, prognosis, and response to treatment [2, 3].

The most important etiological factors are common to all locations and include smoking, chronic alcoholism, poor oral hygiene, chewing betel leaves, and more recently documented, inhalation of E-cigarette vapors and human papillomavirus (HPV) infection, which imprint certain prognostic features [4].

The evolution is mainly loco-regional. Treatment failures are primarily due to recurrences or local or regional evolution (70%) and rarely to metastases (30%), which emphasizes the importance of obtaining local control.

Traditionally, establishment the treatment considers only the *clinical* prognostic factors – category tumor (T), lymph node (N) –, staging according to *American Joint Committee on Cancer* (AJCC), *pathological* prognostic factors – pTNM – and performance status, so that patients in the same stage category benefit from the same therapeutic strategy [5]. However, due to the molecular pathways responsible for the still incompletely elucidated malignant phenotype, offering similar treatments to patients in identical stage categories is associated with significantly different prognostic outcomes [6] (Figures 1 and 2). The therapeutic index could be improved by clinical integration and adaptation of the therapeutic strategy to precision biomarkers that express the genetic differences and biological behavior of tumors (Figures 3–5). The first and most notable example, the expression of p16 protein, surrogate marker of *HPV* status in oropharyngeal cancers, defines a clinical entity with a favorable prognosis, it being the most significant prognostic biomarker, which, according to statistical power, exceeded the *traditional* prognostic factors (T, N category). Differences in response to treatment have paved the way for personalized treatments based on the genetic fingerprint of the tumor [2, 7, 8].

In the multimodal treatment of head and neck cancers, radiotherapy (RT) plays a central role as an alternative to surgery in early stages, accompanied by chemotherapy as a component of organ preservation strategies in loco-regionally advanced laryngeal and hypopharyngeal tumors and postoperatively to eradicate the microscopic disease spread locally and regionally [9–11].

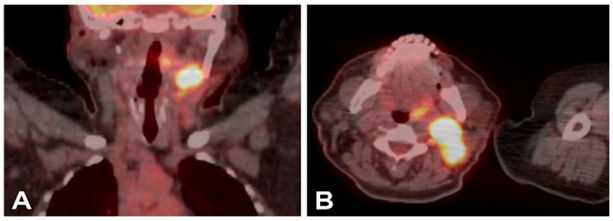

Figure 1 – ***Female patient, 66-year-old, non-smoker, presents with left base of tongue SCC: (A) The pre-therapeutic assessment was completed with PET/CT and evaluation of tissue-based prognostic biomarkers; (B) PET/CT: left base of tongue mass of 23 mm, SUV 10.85; multiple confluent level II left neck nodes 47 mm, SUV 12.84. PET/CT: Positron emission tomography/ Computed tomography; SCC: Squamous cell carcinoma; SUV: Standardized uptake value.***

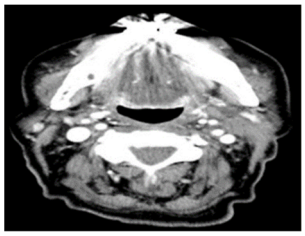

Figure 2 – ***Complete response after concomitant radiotherapy and high-dose Cisplatin chemotherapy, maintained for more than three years of follow-up.***

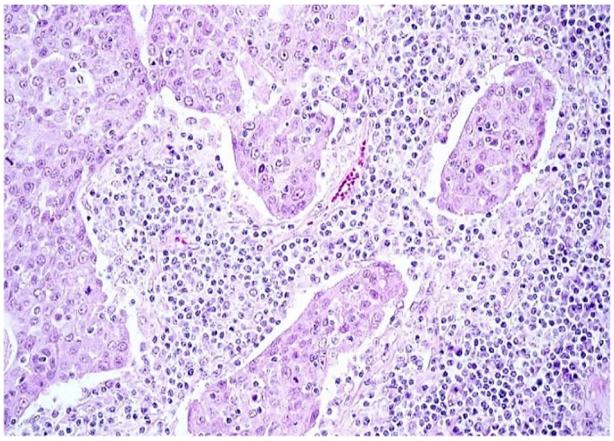

Figure 3 – ***Non-keratinized SCC lymph node metastasis (HE staining, 400×). HE: Hematoxylin–Eosin.***

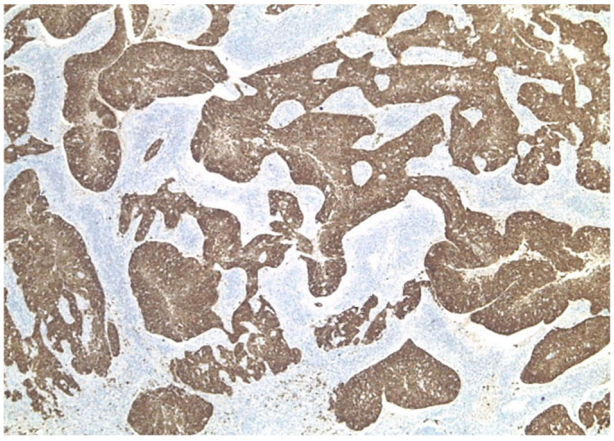

Figure 4 – ***p16 IHC staining of the previous SCC: intense positive and uniform expression advocates for HPV etiology of base of tongue carcinoma (Anti-p16 antibody immunomarking, 40×). HPV: Human papillomavirus; IHC: Immunohistochemical.***

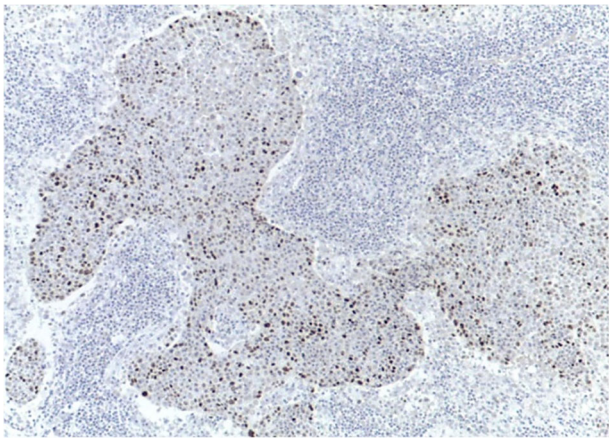

Figure 5 – ***p53 IHC staining: focal positivity of isolated SCC cells defining non-mutant pattern (Anti-p53 antibody immunomarking, 100×).***

Despite the improvement in the quality of RT over the last two decades through introducing of intensity-modulated radiation therapy (IMRT), volumetric-modulated arc therapy (VMAT) and image-guided radiation therapy (IGRT), which allow target volume delineation and precise conformation of the radiation dose to the three-dimensional configuration of the tumor and its extensions, the success of further treatment is limited by local recurrence and late toxicity that significantly alter the patients' quality of life (QoL) [9].

With a 5-year overall survival (OS) of 40–60%, approximately 30% of patients who had exclusive radio-chemotherapy will come back with local recurrences. Tumors are classified as radioresistant if the recurrence is diagnosed after a disease-free interval (DFI) of less than six months [12]. Treatment options are considerably reduced for patients with recurrences or primary tumors in previously irradiated areas. Thus, rescue surgical treatment and HPV status are predictive factors associated with rates of 2-year survival at exceeding 50%. In their absence, the prognosis is reserved, with a median survival rate of six to three months [13, 14].

At the cellular level, the main irradiation target is the deoxyribonucleic acid (DNA), its lesions being largely responsible for cell death induced by irradiation. However, a significant part of the radiation-induced DNA damage will be repaired through the activity of an intricate network comprising signaling proteins and repair pathways. The irradiated cell depends on their functionality. Radio-biologically, compared to normal cells, certain components or repair mechanisms of radiation-induced DNA damage are deficient or aberrantly expressed in tumor clonogens, survival being ensured by the activity of the remaining repair pathways. At the same time, the repair mechanisms are major obstacles in obtaining the local control responsible for the recurrence or tumor evolution but also for potential therapeutic targets [15]. Consequently, tumors with an increased ability to repair DNA damage will be radioresistant, while the suboptimal activity of repair pathways, especially double-stranded ones – the most lethal lesions induced by RT – will have a detrimental effect, endangering cell survival.

In over 90% of cases, head and neck cancers overexpress epidermal growth factor receptor (EGFR) and the most common somatic mutations are of p53. Both are associated with radioresistance of head and neck cancers by amplifying the repair of radiation-induced DNA damage. The HPV

etiology is increasing and the role of p16 in modulating repair of radiation-induced DNA lesions is performed independently of its function in controlling the cell cycle [6, 12, 16].

Aim

The purpose of this review was to elucidate the mechanisms involved in avoiding radiation-induced apoptosis of head and neck cancers mediated by modulating the repair of DNA damage *via* p53, EGFR and p16. The role of DNA damage-associated biomarkers in response to irradiation in clinical practice in the selection of personalized treatments and specifying the prognosis and, finally, the bases of immunotherapy association are presented.

DNA damage response to tumor irradiation – potential therapeutic target in head and neck cancers

Nuclear DNA damage is the ultimate target of irradiation and the main mechanism by which irradiation causes cell death. The energy of ionizing radiation can be stored directly at DNA level, but it is more frequently stored indirectly, involving intermediate reactive products resulting from water radiolysis – free radicals. It is estimated that for the absorbed dose of 1 Gy gamma radiation with low linear energy transfer, approximately 1×10^5 ionizations in the nucleus occur, which in turn cause 850 lesions of the pyrimidine nitrogenous bases, 450 for purine nitrogenous bases, 1000 single-strand breaks (SSBs) and 30 DNA double-strand breaks (DSBs) [17]. The evolution of eukaryotic organisms has been inextricably linked to the ability of defending genome integrity and restoring genetic information lost due to DNA damage from physiological cellular processes: DNA replication, recombination occurring during cell division, free radicals damage resulting from cellular metabolism and ultraviolet radiation exposure. Thus, SSBs will be repaired through *base excision repair* (*BER*), DNA adducts – through *nucleotide excision repair*, and DSBs through *homologous recombination* (*HR*) and *non-homologous end joining* (*NHEJ*) [18, 19]. DSB represents the discontinuity in the phosphodiester backbone of both DNA strands separated by <10 pairs of nitrogenous bases and are the most lethal lesions, responsible for radiation-induced cell death. In conventional fractionated RT, 2 Gy per fraction causes 3000 DNA lesions per exposed cell, 40 DSBs being sufficient to cause cell death [17]. HR ensures high-accuracy repair of DSBs and adducts in a multistage process, with the sister chromatid serving as a matrix for reconstructing the lost DNA sequence, thus limited only to lesions produced in the S or G2 phase. HR is defined by *the invasion of the complementary DNA molecule in search of the homologous sequence* (*synaptic complex*) and the exchange of DNA sequences. Tumor suppressor proteins breast cancer 1 (BRCA1), breast cancer 2 (BRCA2), replication protein A1 (RPA1) and RAD51 have a central role in homologous recombination [20, 21].

By comparison, *NHEJ*, the main mechanism for repairing radiation-induced DSBs, takes place throughout the entire cell cycle. This repair pathway involves simply joining the non-homologous DNA ends, thus increasing the risk of mutation through deletions and inserts. This type of repair involves attaching the Ku70/80 heterodimer to the DNA rupture location, signaling by the DNA–protein kinase (PK) catalytic complex, and activating X-ray repair cross-complementation 4 (XRCC4)–XRCC4-like factor (XLF) responsible for the binding function of the damaged DNA ends [22]. In relation to cell proliferation, DSBs are signaled to cell cycle control checkpoints that maintain the inactive status of cyclin-dependent kinase (CDK) and implicitly delay division until DNA damage is repaired. Therefore, DSBs are signaled by ataxia telangiectasia mutated (ATM) and phosphorylation of H2A histone family member X (H2AX) to γ-H2AX occurs. ATM and checkpoint kinase 2 (CHK2) assign p53 a stable form due to dissociation from murine double minute 2 (MDM2), its negative regulator, with downstream activation of its effector p21. P21 accumulation cancels G1/M cell cycle progression through binding to cyclin E/CDK2 and cyclin D/CDK4/6 complexes. Alternatively, after irradiation, ATM-signaled DSBs activate CHK2 and upstream cell division cycle 25 (CDC25) through phosphorylation in consequence of canceling the progression from the G2/M phase of the cell cycle *via* cyclin B1/CDK1. Similarly, SSBs are recognized by ataxia telangiectasia and RAD3-related (ATR) serine/threonine kinase that binds to the lesion site *via* checkpoint kinase 1 (CHK1), preventing cell cycle progression until DNA damage repair [23].

The regulation of the control points of the G1/S and G2/M cell cycles ensures adequate cell proliferation [12].

Compared to normal cells, tumoral DNA damage response to radiation has fundamentally different features that make these pathways and their proteins an attractive therapeutic target [24]. To summarize, these features are as follows:

DNA repair mechanism malfunction that increases dependence on the remaining repair pathways

Given the role of HR in the remediation of DSB and adducts [interstrand crosslinks (ICLs)], cancers with deficiencies of this pathway will be particularly sensitive to Platinum doublets chemotherapy.

Recovering the constitutional loss of a molecular pathway that is a tumor-specific event and pharmacologically blocking the remaining pathways opens the possibility of exploiting the mechanism of *synthetic lethality* following the example of mutant *BRCA* ovarian cancer – a strategy that has been researched and shown as promising in phase 2 trials – and in ear, nose and throat (ENT) cancers [25, 26].

Selecting patients with HR deficiencies, predictive of the success of treatments with anti-poly [adenosine diphosphate (ADP) ribose] polymerase (PARP) agents, based solely on *BRCA* mutations, is not the only approach. Thus, the predictive biomarkers that denote HR functionality with the possibility of clinical integration are sequencing a panel of genes involved in DNA repair, genomic "scar" analysis associated with loss of repair pathways or identification of a single deficiency through immunohistochemistry (IHC) [27, 28].

DNA replication stress associated with uncontrolled tumor proliferation

Although it is one of the defining features (known as hallmarks of cancer) involved in cancer etiology, at the

same time uncontrolled tumor proliferation is a potential therapeutic target [29]. The determining factors are loss of cell cycle control proteins, overexpressed oncogenes that lead to accelerated tumor proliferation and implicitly to DNA synthesis in the absence of the necessary resources in the form of the four types of deoxyribonucleotides or of specific factors. At a molecular level, uncontrolled tumor proliferation is associated with deficient and erroneous DNA synthesis due to the suboptimal activity of DNA polymerase. Consequently, multiple single-stranded DNA chains are generated, which, after being protected by fixation of the replication protein A (RPA) and signaled by ATR kinase, will be subjected to DNA repair mechanisms. Given that in the absence of ATR and its effector kinase CHK1, single-strand DNA lesions will be converted into double-strand lesions evolving into mitotic catastrophe and cell death, the pharmacological blockage of these pathways is sought. Targeting the defective DNA synthesis associated with uncontrolled replication has the advantage of improving the therapeutic index through the selective activity of these inhibitors at tumor level, while sparing healthy tissues [30].

P53 binding protein (53BP1) and single-strand DNA detection are predictive biomarkers for successful ATR inhibitor treatment and thus surrogate markers of deficient DNA synthesis associated with uncontrolled replication [31].

Increased constitutional levels of endogenous DNA damage

Tumor cells are characterized by constitutionally elevated levels of reactive oxygen species involved in carcinogenesis. The direct consequence is the structurally altered nucleotides, most commonly 8-oxoguanine, caused by mismatch mutations. These DNA lesions can be repaired by the base excision mechanism or can be perpetuated due to tolerance, ultimately contributing to defective DNA synthesis [32, 33].

Predictive biomarkers for DNA damage response to irradiation in head and neck cancers: clinical implications

Although in the treatment algorithm of head and neck cancers RT takes into consideration only traditional prognostic factors (clinical and pathological) and there are no predictive biomarkers to influence the choice of treatment method, some candidates, currently undergoing research, intervene in repairing radiation-induced DNA lesions: p16 status, *p53* gene mutations and EGFR over-expression [6].

Mutational status of p53

In head and neck cancers, *p53* gene mutations are most common, going up to 85% in the case of HPV-negative tumors [34]. The gene carries a tumor suppressor protein activated under conditions of cellular aggression with the role of transcription factor that coordinates the expression of over 100 other genes involved in cell cycle blocking, DNA damage repair, apoptosis – mechanisms meant to protect genome integrity [35].

Tumor protein p53 (*TP53*) mutations have been associated with loco-regional recurrence, decreased survival, and have a predictive significance for response to Platinum doublet chemotherapy [36, 37] (Figure 5).

In the presence of ionizing radiation, DNA–PK and ATM sensor kinases activate p53, resulting in cell cycle blockage followed by DNA repair or apoptosis, depending on the severity of the radiation-induced DNA damage [38]. Significant, irreparable lesions lead to caspase-mediated apoptosis intrinsically, by transcription of B-cell lymphoma-2 (BCL-2) and BH3 proteins or extrinsically, with the participation of tumor necrosis factor (TNF) family receptors, activated in the presence of specific ligands. Conversely, remediable DNA lesions are signaled by ATM, the negative regulatory factor of p53 – MDM2 is inhibited and consequently p53 binds to the p21 promoter. The result of p21 protein activation is CDK2 and CDK4 inhibiting. Retinoblastoma protein (pRb) remains attached to E2F transcription factor 1 (E2F1) by blocking the cell cycle in the G1 phase, during which time radiation-induced DNA damage is repaired [39].

In the presence of *p53* mutations, DSB repair is suboptimal but sufficient to allow the proliferation and selection of tumor clones that will accumulate genetic alterations that give them resistance to treatment. Thus, mutations with *loss of function* prevent cell cycle blockage and radiation-induced apoptosis. Alternatively, *gain of function* mutations, often of the dominant type, determine resistance to radiation-induced cell death by attributing oncogenic properties: sustained DNA repair, invasiveness, uncontrolled proliferation, cancellation of ATM function and cellular metabolism alteration [40].

Given the role of p53 as a mediator of the DNA response to irradiation, establishing the clinical implication of its mutations in head and neck cancers has been investigated extensively.

Studies that have analyzed the prognostic and predictive value for RT response have reached discordant conclusions that are explained by the structural and functional complexity and the multitude of mutations reported. Experimental techniques were variable, from IHC evaluation to partial sequencing of the *p53* gene genome [41, 42]. The classification system proposed by Poeta *et al*. and Lindenbergh-van der Plas *et al*. considers the structural complexity of the p53 molecule and defines the mutations as follows: *disruptive* – with significant alteration of the amino acid sequence at the DNA junction region – *or non-disruptive*. In head and neck cancers that had the RT sequence in multimodal treatment, disruptive *p53* mutations were associated with loco-regional recurrence, decreased OS and free of disease progression, retaining significance after multivariate analysis that included HPV status [43, 44].

p16 status

In head and neck cancers, HPV infection defines an epidemiologically, demographically, and clinically distinct oropharyngeal tumor entity. Infectious etiology of HPV – the high-risk HPV-16 and HPV-18 strains exceed traditional risk factors, in contemporary cases reaching 70% [45, 46]. Often these patients are young, with no history of substance abuse, have significant satellite adenopathy and small tumors, and the response to RT and the survival rate are considerably improved compared to forms with classical toxic etiology (HPV-negative) [47].

In clinical practice, establishing high-risk HPV infectious etiology is mandatory in oropharyngeal cancers and squamous

cell carcinoma (SCC) of unknown primary in upper or middle jugular lymph nodes. Since messenger ribonucleic acid (mRNA) evaluation of E6 and E7 viral oncoproteins is technically challenging it takes place by determining the expression of the cell cycle regulator, the p16 protein, surrogate biomarker of HPV infection, through IHC [48] (Figure 3).

Multiple recent studies confirm the strong association between HPV infection status and p16 overexpression [49].

Viral carcinogenesis is initiated by the E6 and E7 oncoproteins, which cancel the p53 and pRb tumor suppressor proteins, with a role in regulating the progression in cell cycle phases and coordinating DNA repair pathways. The result is the uncontrolled expression of the cyclin-dependent protein kinase inhibitor p16 with genome instability, uncontrolled proliferation, accumulation of chromosomal aberrations and implicit progression to the malignant phenotype [50, 51]. The particular radiosensitivity of HPV-positive cell lines is explained by suboptimal DSB repair mechanisms compared to HPV-negative ones and alteration of the G2/M phase transition of the cell cycle [52, 53].

The role of p16 in modulating the DNA response to irradiation is performed independently of its cell cycle control function. Thus, p16 dislocates cyclin D1, a factor involved in radioresistance in multiple tumor localizations [54–56], from the cyclin D1–CDK4/6 complex that leads to its degradation. The *in vivo* consequence is the deficient anchoring of RAD51 to the damaged DNA site and decreased radiation-induced lesion repair capacity through HR. This data is confirmed by the significant association between HPV-negative status and/or low p16 expression with elevated cyclin D1 levels [56, 57].

Given the favorable response of HPV-positive oropharyngeal tumors to radiochemotherapy, studies are underway to de-intensify therapeutic strategies, primarily to reduce late-onset toxicities that greatly affect the patients' QoL. De-escalation strategies aim to reduce the *radiation dose*, exploring the efficiency of *Cetuximab* as an alternative to Platinum-based chemotherapy in *concomitant radio-chemotherapy* treatment, reducing postoperative *adjuvant chemotherapy* or *RT* (guided by the pathological characteristics of the resection specimen) [58–60].

However, until the certain benefit of these strategies is demonstrated without detrimental effect on local control and survival, HPV status remains a prognostic rather than a predictive biomarker capable of influencing therapeutic decision.

EGFR

EGFR is a transmembrane protein with an activated receptor role in the presence of ligands [epidermal growth factor (EGF) and transforming growth factor (TGF)-α and -β], determining the activation of phosphoinositide 3-kinase (PI3K) and RAS pathways responsible for uncontrolled cell proliferation, apoptosis inhibition and angiogenesis [61]. EGFR is overexpressed in >90% of head and neck cancers, its expression along with that of its ligands being an unfavorable prognostic factor [62] (Figure 6). RT mimics the EGFR–ligand interaction. EGFR activation takes place through phosphorylation and, consequently, determines sustained proliferative signal and survival of tumor cells with cascade activation of RAS and PI3K pathways through the mitogen-activated protein kinase (MAPK) mediator [1 2]. Clinically, this translates to RT failure. In radiation-treated head and neck cancers, EGFR expression determined by IHC is an independent predictive factor for loco-regional recurrence [63, 64].

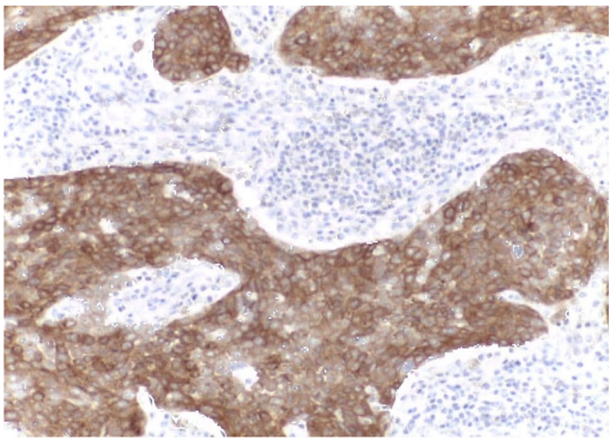

Figure 6 – ***EGFR immunohistochemistry: membrane and cytoplasmic intense positive and uniform staining of tumor cells (Anti-EGFR antibody immunomarking, 200×). EGFR: Epidermal growth factor receptor.***

The high level of expression correlates with the shortening of the DFI in chemotherapy-treated and irradiated naso-pharyngeal, laryngeal and hypopharyngeal cancers [6, 65]. EGFR contributes to the successful repair of radiation-induced DSB and to cell cycle progression by direct interaction with DNA–PK, a protein with a central role in NHEJ repair and by regulating ATM transcription with a role in signaling double-strand lesions [66]. For these reasons, the combination of concomitant radioimmunotherapy with anti-EGFR agents, the first evaluated being the anti-EGFR monoclonal antibody – Cetuximab is a therapeutic strategy that has been received with hope. In 2006, Bonner *et al*. demonstrated improved survival and local control in patients with advanced loco-regional head and neck cancers that were radioimmunotreated with co-administration of Cetuximab *versus* exclusive RT [67].

The benefit was questioned in the RTOG 0522 study in which the addition of Cetuximab to chemoradiotherapy in stage III and IV oropharyngeal cancers did not improve survival, loco-regional and remote control. It is worth mentioning that the status of EGFR expression was not predictive of response to Cetuximab treatment [68].

In 2019, RTOG 1016 sets a *standard of care* in stage III–IV oropharyngeal cancers: IMRT + chemotherapy concomitant with Cisplatin in high dose. Concomitant radioimmunotherapy and Cetuximab had a detrimental effect on survival and loco-regional control with no differences in toxicity rates. These results were also confirmed in the following trials for low-risk and/or HPV-positive patients [69, 70]. For patients with contraindication to concomitant Cisplatin chemotherapy, administration of Cetuximab is standard. The combination of RT with concurrent Cetuximab and Durvalumab in locally advanced head and neck SCC is currently investigated in DUCRO trial [71].

Notably, in studies on tumor cell lines, a significant discrepancy was demonstrated between EGFR expression and EGFR pathway activity, whose surrogate marker is autophosphorylation of the EGFR receptor – the target of

EGFR inhibitory agents. EGFR expression was not associated with EGFR pathway activity, valid on both cell lines and tumor cells, which questions the predictive role of EGFR expression for treatments with anti-EGFR inhibitors. The EGFR inhibitors that were studied, Cetuximab and Erlotinib, demonstrated limited efficiency in the active status of the phosphorylated EGFR/EGFR pathway and for a low number with low levels of the EGFR pathway activity due to additional factors involved in EGFR polymorphisms, mutations in upstream pathways [72].

The results of these studies confirm the importance of predicting which patients will benefit from therapeutic combinations and of identifying new therapeutic targets. Inhibition of the EGFR pathway will most likely depend on the validation of predictive biomarkers.

Future therapeutic perspectives: the combination of radioimmunotherapy and the exploitation of the abscopal and by-stander effects

In addition to its cytotoxic properties, RT intervenes in the immune and tumor microenvironment modulation. The mechanisms by which RT amplifies the antitumor immune response are activation and proliferation of tumor-infiltrating lymphocytes, release of tumor-specific antigens during radiation-induced cell death and, due to sustained inflammatory response, alteration of chemokines with activation of cytotoxic T-lymphocytes and amplification of major histocompatibility complex (MHC) class I, dendritic cell migration [73–75]. It is worth mentioning that head and neck cancers are among the first locations where, in the early 1900s, *the abscopal effect* was reported, clinically defined by the regression of a non-irradiated secondary tumor located at a distance following local irradiation [76]. In clinical practice, this remains a rare phenomenon, but growing in use with the introduction of inhibitors of cytotoxic T-lymphocyte associated protein 4 (CTLA4) or programmed cell death-1 (PD-1)/programmed death-ligand 1 (PD-L1) immune control points, stereotactic RT with highly conformational administration of ablative doses of RT, manipulation of the RT sequence, dose fractionation elements [77–79]. However, the major challenge is identifying specific categories of patients who could benefit the most from radioimmunotherapy association. Several reports have linked biomarkers of DNA repair to the response to immune checkpoint blockade through increased mutational burden and neoantigen load [80]. Alternatively, at the level of the primary tumor, non-irradiated tumor clonogens located adjacently can respond radiobiologically in a similar way to the irradiated ones, with favorable consequences regarding loco-regional control, defining the by-stander effect of RT [81, 82]. Surprisingly, radioresistant tumor cell lines are the ones that benefit, mainly due to the *by-stander effect*, the biomarkers associated with the DNA damage response to irradiation being overexpressed, with the prospect of changing the current approach in the planning process and defining target volumes [83].

Conclusions

In head and neck cancers, RT is a potentially curative therapeutic method when it is adapted and applied to selected categories of patients. The radiobiological effectiveness of irradiation is determined by the ability to generate DNA DSBs, while signaling proteins and repair pathways are aberrantly expressed at the tumor level, showing differences in radiosensitivity. Tumors with an increased repair capacity will be radioresistant. Head and neck cancers overexpress EGFR and the most common somatic mutations are of p53, both favoring radioresistance by amplifying the repair of radiation-induced DNA damage; the HPV etiology of oropharyngeal cancer is increasing and the role of p16 in modulating the repair of radiation-induced DNA damage is done independently of its function in controlling cell cycle. As traditional prognostic factors only partially explain the unpredictable evolution of head and neck cancers, the therapeutic index could be further improved by adapting therapeutic strategies to an association of biomarkers to express the functionality of repair mechanisms.

Conflict of interests

The authors declare that they have no conflict of interests.

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