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LETTERS TO THE EDITOR

PTEN in laryngeal carcinomas

Dear Editor,

The PI3K/AKT/PTEN/m TOR signaling transduction pathway regulates many critical cell functions including cell proliferation, protein synthesis and survival. Concerning carcinogenesis, gene imbalances lead to tumor growth and angiogenesis by deregulating VEGF and hypoxia-inducible factor-1 (HIF-1) expression (Figure 1). *PTEN* (gene locus: 10q23.3-phosphatase and tensin homolog deleted in chromosome 10) is a tumor suppressor gene that is deleted, mutated or epigenetically hypermethylated in a variety of human malignancies. PTEN acts as a negative regulator of this specific pathway. Normal expression of PTEN induces growth suppression by promoting cell cycle arrest. It is also correlated with decreased levels and nuclear localization of cyclin D1 regulated by AKT that positively induces cell cycle.

PTEN downregulation in laryngeal squamous cell carcinoma (LSCC) seems to be a significant genetic event affecting some biological characteristics of the malignancy such as proliferation and invasion of cancer cells, although there are controversial results. Quite recently a study group showed that the DJ-1 protein overexpression - in transfected human LSCC SNU-46 cell line by a retrovirus carrying - inhibited PTEN expression combined with p-AKT/ p-mTOR upregulation [1]. Furthermore, introduction of rapamycin, an inhibitor of mTOR depended signaling pathway leads to proliferation downregulation combined with apoptosis induction in LSCC *in vitro* [2]. Additionally, intergenic noncoding RNAs, such as Homeobox (HOX) transcript antisense RNA (HOTAIR), play an oncogenic role in deregulating the current pathway by significantly overexpressed in LSCC as observed in breast and hepatocellular cancers [3]. This noncoding RNA enhances PTEN hypermethylation which drives to its loss of protein expression.

Besides PTEN promoter gene methylation imbalances, micro-RNAs (miRs) provide new epigenetic mechanisms for PTEN regulation in LSCC. miRs are short, non-coding RNAs consisting of 20-25 nucleotides located at intra- or intergenic regions. Their deregulation in cancer cells due to genetic (mutations, translocations), epigenetic (DNA hypermethylation of tumor suppressor genes, extensive genomic DNA hypomethylation, aberrant histone modification patterns) and transcriptional alterations leads to a loss of miRs-mediated repression of target mRNA. Concerning LSCC, PTEN was identified to be directly regulated by miR-1297 as it happens in Hep-2 cell lines *in vitro*. miR-1297 overexpression promotes cancer cell proliferation, migration and invasion in LSCC, whereas its downregulation affects positively PTEN expression inducing its tumor suppression action [4]. In conjunction to this, another crucial aspect is the effect of PTEN expression in tumor recurrence after surgical resection combined or not with radiotherapy. A study group showed that loss of PTEN and p27 protein expression is correlated with high recurrence rates after CO_2 laser resection in LSCC – especially of stage T1 glottic carcinoma patients [5]. In conclusion, PTEN gene silence disorganizes the PI3K/AKT/PTEN/mTOR signaling transduction pathway in LSCC, affecting potentially the response rates to specific targeted therapeutic agents and the prognosis in those patients.

References

1. Wang B, Qin H, Wang Y et al. Effect of DJ-1 overexpression on the proliferation, apoptosis, invasion and migration of laryngeal squamous cell carcinoma SNU-46 cells through PI3K/AKT/mTOR. Oncol Rep 2014; 32:1108-1116.
2. Li R, Wang R, Zhai R, Dong Z. Targeted inhibition of mammalian target of rapamycin (mTOR) signaling pathway inhibits proliferation and induces apoptosis of laryngeal carcinoma cells in vitro. Tumori 2011;97:781-786.

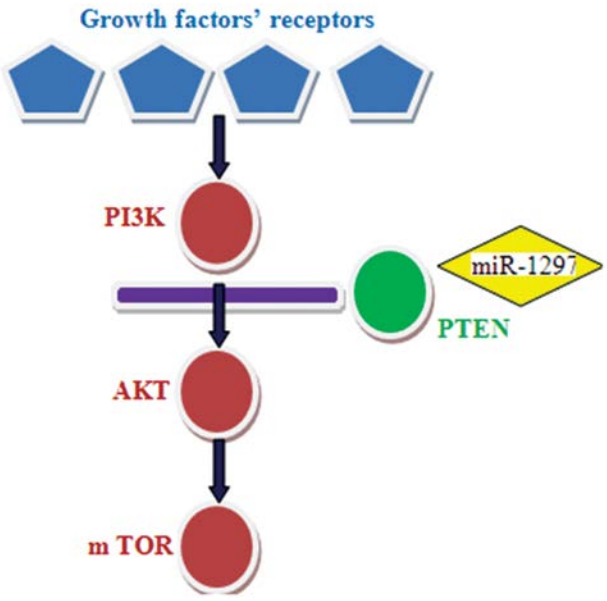

Figure 1. The PI3K/AKT/PTEN/m TOR signaling transduction pathway.

3. Li D, Feng J, Wu T et al. Long intergenic noncoding RNA HOTAIR is overexpressed and regulates PTEN methylation in laryngeal squamous cell carcinoma. Am J Pathol 2013;182:64-70.
4. Li X, Wang HL, Peng X, Zhou HF, Wang X. miR-1297 mediates PTEN expression and contributes to cell progression in LSCC. Biochem Biophys Res Commun 2012;427:254-260.
5. Yang JQ, Liang Z, Wu M, Sun YM, Liu HX. Expression of p27 and PTEN and clinical characteristics in early laryngeal squamous cell carcinoma and their correlation with recurrence. Int J Clin Exp Pathol 2015;8:5715-5720.

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Re-affirming the necessity of sending even minor surgical specimens for histopathology: a case of inverted papilloma of the adenoids

Dear Editor,

We recently came across the case of a 7.5 year-old girl from a remote Greek island, referred to our Department due to persistent otitis media with effusion in the right ear and snoring. Endoscopic assessment revealed enlarged but symmetrical adenoidal pad. The child underwent cold-steel adenoidectomy, and right grommet insertion under general anesthesia. The histopathology of the excised adenoids demonstrated the presence of inverted papilloma in as much as 40% of the specimen volume, characterized by predominantly squamous neoplastic epithelium with parakeratosis and at lower parts mitoses and nuclear irregularities consistent with reactive atypia (Figures 1a & 1b). The molecular analysis of the specimen revealed abundant presence of HPV type 16, along with elevated Ki67 expression. Since the case represented virtually unchartered waters, it was decided to be followed-up in a manner similar to sino-nasal inverted papilloma.

The child returned from the island three months postoperatively, and underwent MRI scan of the paranasal sinuses, which was clear. She further underwent a scheduled examination under anesthesia of her nasopharynx six months after the initial procedure. There was recurrence of friable adenoidal tissue in the area of the left posterior choana, which was completely removed by Jurasz cutting forceps and meticulously cauterized by suction-diathermy. Separate biopsies were also taken from all other ar-

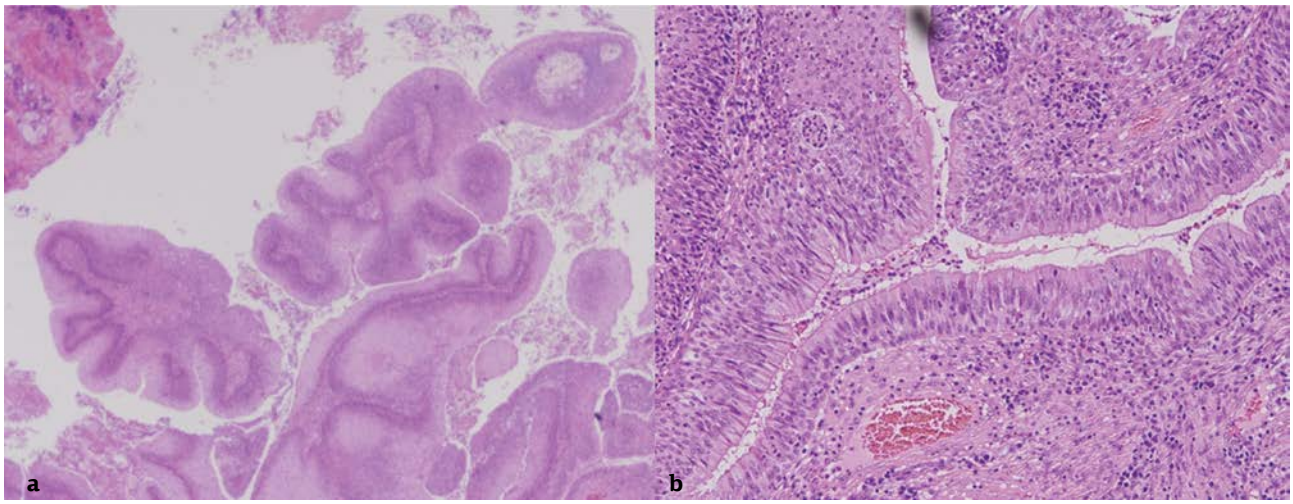

Figure 1. a: Microphotograph of inverted papilloma of the adenoids. Normal adenoidal tissue (upper quadrant), as opposed to respiratory epithelium (main photographic plane;H&E x10). **b:** greater magnification of the surgical specimen demonstrating in detail the tumor architecture (H&E x20).

eas of the nasopharynx. Histopathology was suggestive of inverted papilloma in the left posterior choana, bearing exactly the same characteristics as before, and was negative in the other biopsied areas. The child is followed-up every six months; one year after the second operation, she remains free of recurrence.

The ectodermally derived Schneiderian mucosa gives rise to an extremely varied collection of benign and malignant neoplasms [1]. Inverted papillomas are the most frequent type of sinonasal papillomas, with a propensity for recurrence, probably due to incomplete excision [2,3], and a risk of synchronous malignancy, or malignant transformation of 10 and 2%, respectively. A causal relationship between HPV and the pathogenesis and progression of inverted papilloma has been posited since the 1980s [2], with HPV currently considered as being the leading co-factor in this pathogenesis [1].

Regarding the presence of HPV DNA in children with benign adeno-tonsillar hyperplasia, Mammas et al. reported a respective incidence of 9.4% in tonsils and 7.1% in adenoids, without any further data regarding the respective recurrence rates [4]. Our case demonstrated local recurrence at six months postoperatively following routine adenoidectomy, and complete disease control after targeted and meticulous excision of the adenoidal re-growth. It also demonstrated the clinical value of sending any surgical specimen for histopathology, without which the real reasons for any disease recurrence could remain obscured.

In conclusion, inverted papilloma of the adenoids represents a probably under-diagnosed entity in children, closely related to HPV infection. It may account for a proportion of cases in which adenoidal re-growth occurs, but is successfully managed with complete surgical excision. The necessity of sending even minor surgical specimens for histopathologic examination, even if they come from populations which are considered otherwise healthy, is also clearly demonstrated.

References

1. Batsakis JG, Suarez P. Schneiderian papillomas and carcinomas: a review. Adv Anat Pathol 2001;8:53-64.
2. Govindaraj S, Wang H. Does human papilloma virus play a role in sinonasal inverted papilloma? Curr Opin Otolaryngol Head Neck Surg 2014;22:47-51.
3. Jenko K, Kocjan B, Zidar N et al. In inverted papillomas HPV more likely represents incidental colonization than an etiological factor. Virchows Arch 2011;459:529-538.
4. Mammas IN, Sourvinos G, Michael C, Spandidos DA. Human papilloma virus in hyperplastic tonsillar and adenoid tissues in children. Pediatr Infect Dis J 2006;25:1158-1162.

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Place of bevacizumab in radiation-induced edema and necrosis

Dear Editor,

Radiation necrosis is a delayed rare but serious complication causing morbidity and mortality. Radionecrosis is seen in 5-15% in patients undergoing brain radiotherapy (RT). The incidence of this complication is reported to be increased due to higher doses of RT and concurrently administered chemotherapy [1,2].

In recent years, vascular endothelial growth factor (VEGF) has been shown to play a role in the pathogenesis of brain radionecrosis. VEGF is the key mediator of angiogenesis and a potent endothelial cellular growth stimulator. In addition, VEGF is also known to be a vascular permeability factor since it increases vascular leak. Necrosis of tissue increases the production of VEGF due to hypoxia. and with increase of necrosis VEGF production also increases. Therefore, it is reported that inhibition of angiogenesis, thus VEGF activity, may play a crucial role in the prevention of brain radionecrosis. Bevacizumab is one of angiogenesis inhibitors. In a review by Lubelski et al. [3] benefits, costs and the complications encountered during bevacizumab therapy were analyzed in high-grade glial tumors with radionecrosis. They reported that bevacizumab reduced neurologic symptoms and radiologically decreased cerebral edema, although it caused severe complications at high rates. Wang et al. [4] studied the effectiveness and safety of adjuvantly given bevacizumab after cyberknife therapy in metastatic patients with extensive cerebral edema. They found clinical and radiological improvements, while none of the patients developed recur-

rent edema and radiation necrosis. Tye et al. [2] conducted a meta-analysis of 16 studies including bevacizumab therapy in radionecrosis. Bevacizumab has been most commonly used in glioblastoma (31%) followed by anaplastic glioma (14%) and metastatic brain tumors (15%). Overall, pre- and post-treatment radiological evaluation revealed a median decrease in T1 contrast enhancemet of 63% and a 59% median decrease in T2/FLAIR signal [2].

In conclusion, controlling of edema following radiation may limit the subsequent development of necrosis. With its mechanism of action, bevacizumab may be a promising treatment modality in decreasing brain radionecrosis and edema. However, although this may be the beginning of a new era in the treatment of brain radionecrosis, we are still far from providing appropriate treatment for each patient. More importantly, identifying the group of patients thay would be benefited from this treatment would provide protection against drug-induced toxicity as well as cost effectiveness. More studies need to be conducted about this subject.

References

1. Cihan YB, Uzun G, Yildiz S, Dönmez H. Hyperbaric oxygen therapy for radiation-induced brain necrosis in a patient with primary central nervous system lymphoma. J Surg Oncol 2009;100:732-735.
2. Tye K, Engelhard HH, Slavin KV et al. An analysis of radiation necrosis of the central nervous system treated with bevacizumab. J Neurooncol 2014;117:321-327.
3. Lubelski D, Abdullah KG, Weil RJ, Marko NF. Bevacizumab for radiation necrosis following treatment of high grade glioma: a systematic review of the literature. J Neurooncol 2013;115:317-322.
4. Wang Y, Wang E, Pan L et al. A new strategy of CyberKnife treatment system based radiosurgery followed by early use of adjuvant bevacizumab treatment for brain metastasis with extensive cerebral edema. J Neurooncol 2014;119:369-376.

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Composite hairy cell leukemia and chronic lymphocytic leukemia

Dear Editor,

The diagnosis of composite hairy cell leukemia (HCL) and B-chronic lymphocytic leukemia (CLL) is rare and, to the best of our knowledge, only few cases were reported in the English language literature [1-5]. In most cases simultaneous manifestation of HCL and B-CLL was reported [1-4] whereas in one case the diagnosis of coexisting HCL was made 17 years after an initial diagnosis of B-CLL [5]. We report a case which emphasizes the benefit of a combined morphological and immunophenotypic approach to the diagnosis of composite HCL and B-CLL.

A-69-year-old Caucasian man was referred to the Hematology Department, University Hospital of Ioannina, for investigation of persistent pancytopenia. The patient's medical history consisted of Rai 0 B-CLL with normal 3 years ago. Physical examination, CT-imaging studies and routine serum laboratory tests were within normal limits. Bone marrow aspiration resulted in dry tap.

Flow cytometric immunophenotyping performed on peripheral blood revealed two populations. The first population accounting for 30% of the total B-cells was positive for CD19, CD20, CD25, CD11c, CD79b, FMC7 and HLA-DR and negative for CD103 and CD10. The second population accounting for 16% of the total B-cells was positive for CD5, CD23, CD19 and HLA-DR and negative for CD79b and FMC7.

Bone marrow biopsy was performed and histological examination of hematoxylin-eosin (HE) stained tissue sections revealed: a) diffuse interstitial infiltration by medium-sized lymphoid cells with abundant clear cytoplasm and oval or cleaved nuclei; and b): nodular and interstitial infiltration by small lymphoid cells (Figure 1a).

Immunohistochemical studies on formalin-fixed, paraffin-embedded bone marrow sections showed that: a) the medium-sized lymphoid cells were positive for CD20 (strongly), Annexin A1, DBA44 (CD72), CD25 and CD11c and negative for CD5, CD23, ZAP70, BCL6, CD10, CD138, CD103, CD123, TRAP and cyclin D1, and that b): the small lymphoid cells were positive for CD5, CD23, ZAP70 and CD20 (weakly) and negative for BCL6, CD10, CD138, CD103, CD123, CD25, DBA44 (CD72), TRAP, Annexin A1 and cyclin D1 (Figure 1b-i). On the basis of the morphological, immunohistochemical and flow cytometry findings, taken together, the diagnosis of a composite HCL and B-CLL was made. Pentostatin 4mg/m^2 every other week was applied for 12 cycles resulting in com-

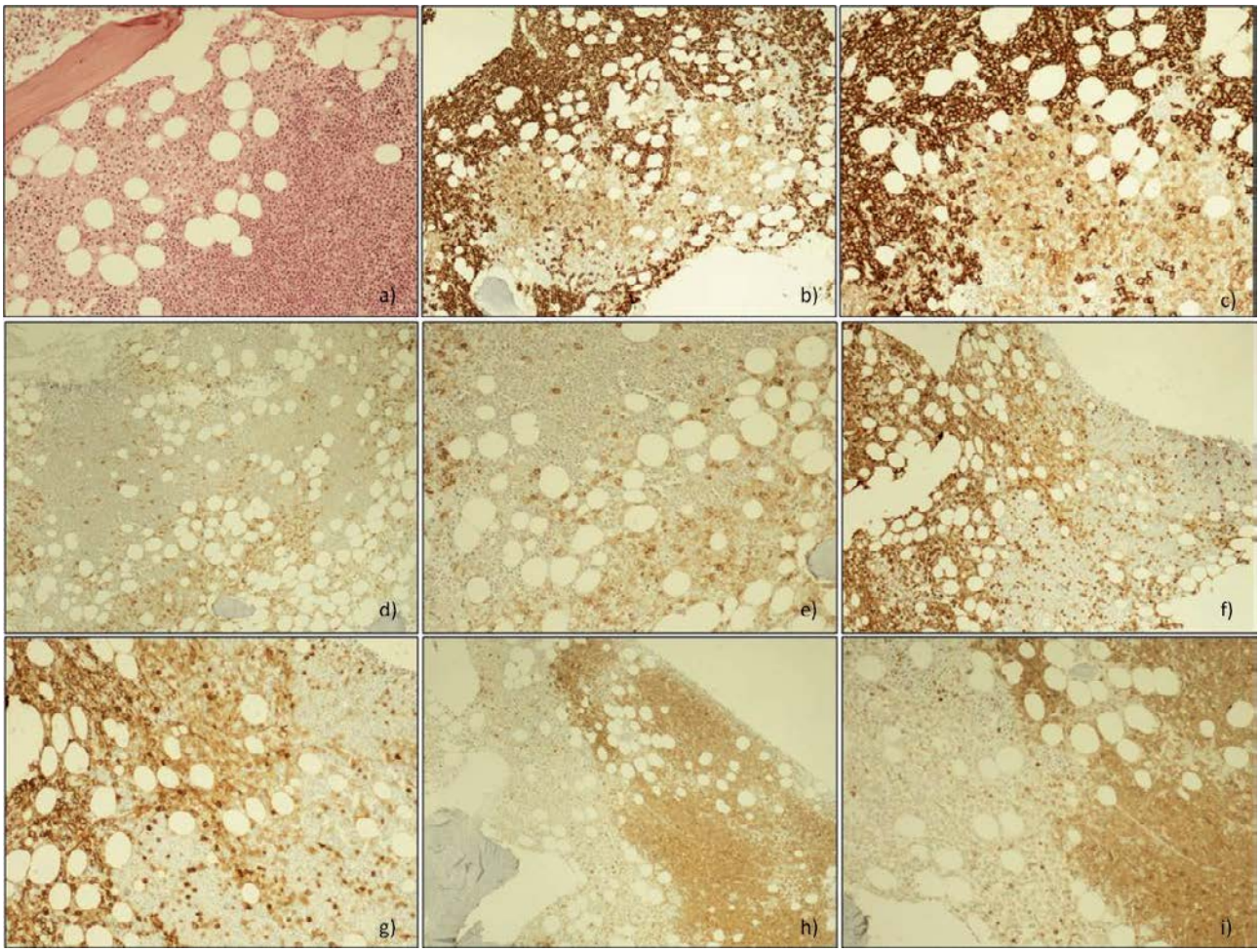

Figure 1. a) infiltration of the bone marrow by a population of medium-sized lymphoid cells with abundant clear cytoplasm and a population of small lymphoid cells (hematoxylin-eosin x200). **b** and **c)** immunohistochemical detection of CD20 in the population of medium-sized lymphoid cells (x100 and x200). **d** and **e)** immunohistochemical detection of DBA44 (CD72) in the population of medium-sized lymphoid cells (x100 and x200). **f** and **g)** immunohistochemical detection of Annexin-A1 in the population of medium-sized lymphoid cells (x100 and x200). **h** and **i)** immunohistochemical detection of ZAP70 in the population of small lymphoid cells (x100 and x200).

plete remission of HCL. At last follow-up 12 months after starting the therapy, the patient was asymptomatic with mild lymphocytosis. At that time, histological examination of the bone marrow revealed nodular infiltration by small lymphoid cells which were positive for CD5, CD23, ZAP70 and CD20 (weakly) and negative for CD25, DBA44 (CD72) and Annexin A1. These findings are consistent with residual B-CLL without evidence of residual bone marrow infiltration by HCL.

In conclusion, one year after initial diagnosis and after 12 cycles of pentostatin, histological and immunohistochemical examination of the bone marrow revealed persistence of B-CLL without residual HCL. Our findings are comparable to previous data showing persistence of B-CLL without residual HCL after completing treatment of patients with composite HCL/B-CLL [1,2] whereas, in another study, a steady increase in the bone marrow HCL component at the expense of the CLL component was observed [5].

References

1. Liptrot S, O' Brien D, Langabeer SE et al. An immunophenotypic and molecular diagnosis of composite hairy cell leukaemia and chronic lymphocytic leukaemia. Med Oncol 2013;30:692-696.
2. Gine E, Bosch F, Villamor N et al. Simultaneous diagnosis of hairy cell leukemia and chronic lymphocytic leukemia/small lymphocytic lymphoma: a frequent association, Leukemia 2002;16:1454-1459.
3. Sokol L, Agosti SJ. Simultaneous manifestation of chronic lymphocytic leukemia (CLL) and hairy cell leukemia (HCL). Am J Hematol 2004;75:107-109.
4. Sanchez ML, Almeida J, Gonzalez D et al. Incidence and clinicobiologic characteristics of leukemic B-cell chronic lymphoproliferative disorders with more than one B-cell clone. Blood 2003;102:2994-3002.

5. Brown SA, Phillips J, Ahsan G, Slater NG. Coexistent hairy cell leukaemia and chronic lymphocytic leukaemia. Leuk Lymph 1998;30:203-209.

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Topoisomerase IIa expression in laryngeal and oral carcinomas: is it a reliable prognostic molecular marker?

Dear Editor,

Topoisomerases are a class of nuclear enzymes that affect the topological structure of DNA. The main members of the family are topoisomerase I (Topo I, gene location 20q11), topoisomerase IIa (Topo IIa) (gene location 17q21), and topoisomerase IIb (Topo IIb, gene location 3p24). Topo IIa and b isoforms' combined action of temporarily cutting and rejoining the DNA double helix, allowing also winding and unwinding of the DNA double strand, is a critically important molecular mechanism for replication, transcription, and repair of chromosome structure. Topo IIa, with a molecular weight of 170 kd, is expressed in proliferating cells in late S phase, with a peak in G2-M phases, where it is believed to be the primary mediator of chromosome condensation. In contrast, Ki 67 protein is expressed in all cell cycle phases (not in Go arrest phase). Topo IIa gene deregulation mechanisms include amplification and also deletion or epigenetic silence (promoter hyper-methylation). Inhibition of topoisomerases promotes cell death, and for this reason they are targets for specific chemotherapy. Many clinical studies have shown that adjuvant chemotherapy strategies, which include anthracyclines (doxorubicin, epirubicin) in conjunction with fluorouracil and cyclophosphamide or carboplatin/ paclitaxel, are most effective, especially in handling patients with breast cancer and other gynaecologic malignancies, such as endometrial or ovarian cancer, respectively [1].

Only limited published data exists concerning the clinical value of Topo IIa expression in oral and laryngeal squamous cell carcinomas (SCC). A study group based on comparative immunohistochemistry and fluorescence *in situ* hybridization (combined Topo IIa/CEP 17) analyses showed that expression of the protein was found to be associated with tumor de-differentiation and advanced tumor T stage in laryngeal SCC. However, the expression of Topo II-α protein was not identified to be associated with Topo II-α amplification in them, although it was found to positively correlate with chromosome 17 aneuploidy. So, aberrant expression and chromosome 17 aneuploidy induced development and progression in those carcinomas, indicating that targeting Topo II-α may provide an important therapeutic strategy [2]. Similarly, another significant study concluded that high Topo II-alpha expression may be a useful indicator of tumor aggressiveness and poor outcome in laryngeal SCC [3]. Additionally to those results, a study group investigated the role of its epigenetic alterations in radio-resistance suggesting that the hypermethylation of TOPO2A might be involved in poor radiotherapy response rates [4]. In conjunction to those results, Topo IIa expression seems to be involved also in the progression and metastatic expansion in oral SCC, especially associated with lymph node metastasis [5]. Based on all of these previous referred studies, Topo IIa gene and protein alterations is a very promising field for discriminating patients by their genetic profile and applying chemo-targeted inhibition agents, such as anthracyclines.

References

1. Tsiambas E, Alexopoulou D, Lambropoulou S, Gerontopoulos K, Karakitsos P, Karameris A. Targeting topoisomerase IIa in endometrial adenocarcinoma: a combined chromogenic in situ hybridization and immunohistochemistry study based on tissue microarrays. Int J Gynecol Cancer 2006;16:1424-1431.
2. Feng Y, Zhang H, Gao W et al. Expression of DNA topoisomerase II-α: Clinical significance in laryngeal carcinoma. Oncol Lett 2014;8:1575-1580.
3. Shvero J, Koren R, Shvili I, Yaniv E, Sadov R, Hadar T. Expression of human DNA Topoisomerase II-alpha in squamous cell carcinoma of the larynx and its correlation with clinicopathologic variables. Am J Clin Pathol

2008;130:934-939.

4. Kim JS, Kim SY, Lee M, Kim SH, Kim SM, Kim EJ Radioresistance in a human laryngeal squamous cell carcinoma cell line is associated with DNA methylation changes and topoisomerase II α. Cancer Biol Ther 2015;16:558-566.
5. Oliveira-Costa JP, Oliveira LR, da Silveira GG, Soave DF, Soares FA, Ribeiro-Silva A. Topoisomerase expression in oral squamous cell carcinoma: relationship with cancer stem cells profiles and lymph node metastasis. J Oral Pathol Med 2012;41:762-768.

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