ANTICANCER RESEARCH *41*: 2773-2779 (2021)
doi:10.21873/anticanres.15058

Review

Impact of Topoisomerases Complex Deregulation on Head and Neck Carcinoma Genomic Instability

EFTHYMIOS KYRODIMOS[1], ARISTEIDIS CHRYSOVERGIS[1], NICHOLAS MASTRONIKOLIS[2], EVANGELOS TSIAMBAS[3], VASILEIOS RAGOS[4], DIMITRIOS PESCHOS[5], DESPOINA SPYROPOULOY[6], PAVLOS PANTOS[1], ATHANASIOS NIOTIS[7] and VASILEIOS PAPANIKOLAOU[1]

[1]*First Department of Otorhinolaryngology, Head and Neck Surgery, Hippocration Hospital, National and Kapodistrian University of Athens, Athens, Greece;*
[2]*Department of Otorhinolaryngology, Medical School, University of Patras, Patras, Greece;*
[3]*Department of Cytology, Immunocytochemistry & Molecular Biology Unit, 417 VA Hospital, Athens, Greece;*
[4]*Department of Maxillofacial Surgery, Medical School, University of Ioannina, Ioannina, Greece;*
[5]*Department of Physiology, Medical School, University of Ioannina, Ioannina, Greece;*
[6]*Department of Radiation Oncology, Medical School, University of Patras, Patras, Greece;*
[7]*Department of Surgery, 417 VA Hospital, Athens, Greece*

Abstract. *Head and neck carcinoma (HNC) comprises a variety of pathological entities. Among them, squamous cell carcinoma (SCC) is histo-pathologically prominent. Specific malignancies, such as nasopharyngeal carcinoma (NPC) arise also from the same anatomical region. In all of them, genomic instability (GI) is implicated not only in the early stages of epithelial malignant transformation, but also in the aggressiveness of the corresponding phenotypes. Among the molecules that are frequently deregulated in solid malignancies including HNCs, topoisomerases (Topo) are of increased significance due to their involvement in DNA topological, structural, and functional stability. The main members are Topo I (20q11), Topo II alpha (17q21) and Topo IIb (3p24). In the current article, we describe the mechanisms of Topo I and Topo IIa deregulation leading to GI in a variety of HNCs. Furthermore, novel data regarding the corresponding targeted therapeutic strategies are presented.*

Correspondence to: Evangelos Tsiambas, Cytopathologist, MD, MSc, Ph.D., Immunocytochemistry & Molecular Biology Unit, 417 VA Hospital,17 Patriarchou Grigoriou E΄ Street, Ag. Paraskevi, 153 41 Athens, Greece. Tel: +30 6946939414, e-mail: tsiambasecyto@yahoo.gr

Key Words: Head, neck, carcinoma, genome, instability, topoisomerase, review.

Concerning solid malignancies, cancerous genomes demonstrate two main types of instability: gross chromosomal instability, including polysomy, monosomy or aneuploidy, and specific gene imbalances including all of the numerical or structural changes (mutations, deletions, translocations, and amplifications) detectedon specific chromosomal loci (1-3). These mechanisms of molecular deregulation lead to dysfunctional and aberrant protein production driving epithelial cells to neoplastic and finally malignant transformation (4, 5). Genetic events that affect significantly signal transduction pathways, cell cycle regulation, proliferation/apoptosis mechanisms, DNA stability, and damage response are mediated by a broad spectrum of genes (6-8). Among them, topoisomerases (Topo) are of great importance due to their implications in DNAtopological structural and functional stability (9). In the current article, we describe Topo I and Topo IIa mechanisms of deregulation in a variety of head and neck carcinomas (HNCs) including oral squamous cell carcinoma (OSCC), laryngeal squamous cell carcinoma (LSCC), and specific malignancies such as nasopharyngeal carcinoma (NPC) arising also from the same anatomical region. Furthermore, we present novel data regarding the corresponding targeted therapeutic strategies.

Topoisomerases: Gene & Protein Structure and Function

Topoisomerases act as nucleic acid enzymes that modify DNA topological structure. Topoisomerase I (gene location 20q11), Topoisomerase II alpha (Topo IIa gene location at 17q21) and

Topoisomerase IIb (gene location at 3p24) represent the main members of the topoisomerase super-family (10) (Figure 1). Concerning their specific actions, Topo I, Topo IIa, and b isomers cut and rejoin temporarily the DNA double helix, enhancing also DNA double strand winding and unwinding. In fact, this is a critically significant molecular mechanism regulating replication, transcription, and repair of chromosome structure (11). Topo IIa has a molecular weight of 170 kDa. Its highest expression is observed in proliferating cells – especially in late S phase-with a peak in G2-M, promoting normal chromosome condensation (12). Based on cell cycle phase-dependent expression, Topo IIa recognizes more specifically actively proliferating cells than Ki-67. For this reason, it is considered a reliable marker of proliferation in a variety of solid malignancies (13-19). Topo I has a molecular weight of 91 kDa and consists of 765 amino acids. It is involved in the unwinding and uncoiling of supercoiled double helix DNA by transiently cleaving one of the two strands. This mechanism provides rotation of one strand over the other and rejoining of ends of the cleaved strand (20). Furthermore, in contrast to Topo II, Topo I is not an ATP-depended enzyme (21). Additionally, Topo II a and b isoforms participate in DNA's molecule stereotype domain stability by passing one DNA double-stranded segment in a second one (22-25).

Regarding topoisomerase inhibition strategies, new agents have been developed and applied to promote cancer cell death such as targeted specific chemotherapy. Regarding also the efficacy of Topo IIa inhibition, many clinical studies have shown that adjuvant chemotherapy strategies, which include anthracyclines (doxorubicin) and podophyllotoxins (etoposide) in conjunction with fluorouracil and cyclophosphamide or carboplatin/paclitaxel are most effective, especially in handling patients with breast cancer and other gynecologic malignancies, such as endometrial or ovarian cancer (26-28). Furthermore, since Topo I down-regulation enhances cell death by leading to DNA damage. It is a target for specific chemotherapy (camptothecin, topotecan, irinotecan) in solid malignancies (29-31).

Topoisomerases in OSCC

Among HNSCCs, OSCC is characterized by a broad spectrum of genomic imbalances, including gross chromosomal alterations, such as polysomy/aneuploidy, and specific gene aberrations. Development and progression of OCSSC is a multistep procedure triggered by a variety of causes, such as chronic tobacco, alcohol, andbetel quid consumption. Furthermore, persistent viral infections - especially high-risk human papilloma virus (HR-HPV) – are also involved in the carcinogenic process (32, 33). HPV positivity is observed in significant proportions of premalignant lesions in the oral mucosa (intraepithelial dysplasias), such as oral atrophic lichen planus (OALP). HPV persistent infection in these cases leads to a progressive transformation of the normal mucosa to malignant tissue. A study group investigated the role of Topo IIa expression and HPV DNA existence in OALP tissues. They reported a strong correlation between HPV-infection and subgroups of atrophic OLP. Topo IIα over-expression was also related to HPV positivity in these lesions (34). Similarly, another study group focused on the role of Topo IIa, Ki-67 and cytokeratin-19 (CK-19) in OALP pre-malignant lesions. The latter molecule represents an intra-cytoplasmic, intermediate filament protein. They showed that Topo IIa should be considered a reliable proliferation and apoptotic marker in OALP lesions indicating also a progressive malignant transformation of the oral mucosa (35). Concerning the influence of Topo IIa on proliferation and apoptosis in oral pre-neoplastic lesions and OSCCs, another protein analysis showed that Topo IIa demonstrated progressively increased expression in hyperplastic, dysplastic, and OSCC tissues (36). Interestingly, Topo IIa expression seems to be involved also in the progression and metastatic expansion of OSCC, especially correlated also to lymph node metastasis (37). Besides Topo IIa, cyclooxygenase (COX)-2 also seems to be critical in oral mucosa carcinogenetic process. A study group co-analyzed cyclooxygenase (COX)-2 and Topo IIa expression in precancerous and cancerous lesions of the oral mucosa. They concluded that patients who demonstrated elevated protein expression levels of both COX-2 and Topo IIa showed poor prognosis (38).

Topoisomerases in LSCC

LSCC represents the most frequent malignancy of this anatomical region (39). Oncogenic high-risk human papillomavirus (HR-HPV) persistent infections, combined or not with chronic alcohol and tobacco consumption, are well established significant pathogenetic factors for LSCC development and progression (40, 41). Because increased cell proliferation is a significant marker for evaluating development and progression rates of carcinogenesis, over-expression of Topo IIa and Ki-67 (cytogenetic band: 10q26.2) in LSCC appears to be a very important factor (42-44). Additionally, it has been shown that Topo IIa deregulation seems to be an early genetic event in LSCC and the Topo IIa-to-Ki-67 ratio could be used as a sensitive proliferation marker (45). Topo IIa gene deregulation mechanisms (amplification) and chromosome 17 status (normal diploid or aneuploid) have been shown to be implicated in aggressive LSCC phenotypes, but only gross chromosome abnormalities in the expression patterns of Topo IIa (46). Another critical observation is that Topo IIa over-expression is associated predominantly with the grade of the examined tumors (47). Concerning the impact of Topo I aberrant expression in LSCC, there are very limited published data. Extensive mRNA–based quantitative RT-PCR analyses suggested that normalized Topo I/G6PDH

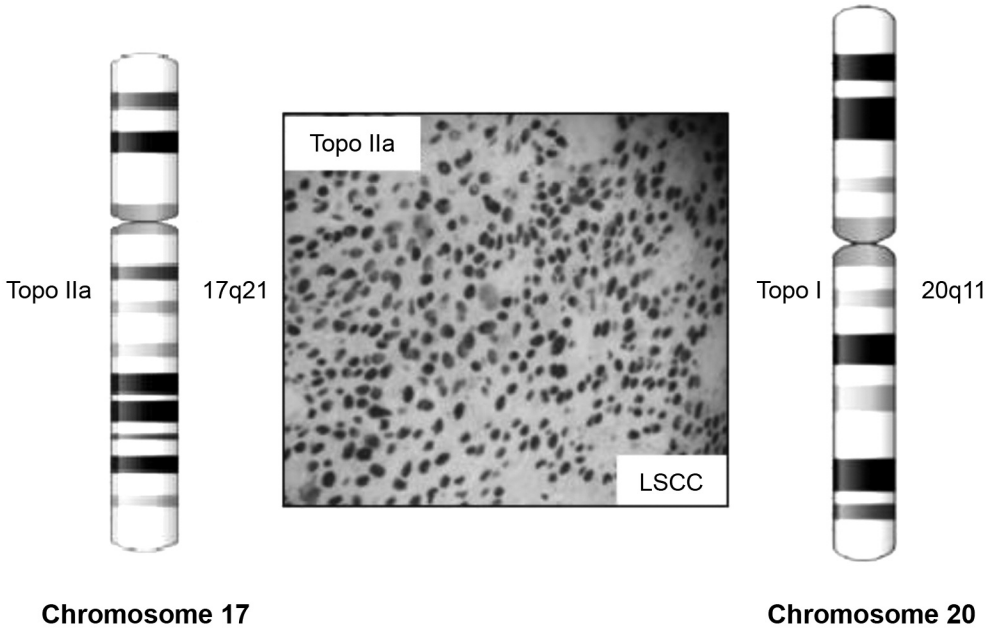

Figure 1. *Schematic of chromosomes 17, and 20 containing the Topo IIa & Topo I genes. Topo IIa protein over-expression in a case of laryngeal squamous cell carcinoma (LSCC). Note the prominent dark, black/grey nuclear staining pattern (center) (Immunohistochemistry technique, DAB chromogen, original magnification: 100×).*

mRNA ratios were strongly associated with Topo I/ porphobilinogen deaminase (PBGD) expression, as has been observed in colorectal tumors but not in pharyngeal/laryngeal tumors (48). In combination with the previous observations, other similar analyseshave shownthat aberrant expression of Topo IIa, but not Topo I, may be a potentially strong indicator of tumor aggressiveness and poor outcome in LSCC, as in esophageal carcinoma (49).

Topoisomerases in NPC

NPC represents a unique, aggressive histo-type entity in the HNC family of malignancies. NPC's tissue origins are the nasopharyngeal epithelia. The malignancy is characterized by a highly invasive and distal metastatic potential negatively affecting patients' prognosis. Concerning the pathogenetic factors implicated in its rise and progression, Epstein-Barr virus's (EBV) persistent infection is prominent. EBV's oncogenic activity in the target cells results to an aberrant expression of specific critical proteins including LMPs and EBNA1 modified by endogenous EBV micro-RNAs (miRs) (50, 51). Concerning Topo IIa aberrant expression in NPC, immunohistochemistry-based studies reported that its over-expression is significantly associated with tumor aggressiveness (advanced stage) and may modify the response to novel target chemo-therapeutic regimens (52). A multi-gene functional molecular analysis revealed a significant number of genes implicated in NPC development and progression (179 up-regulated/238 down-regulated). Topo IIa deregulation is part of specific gene signatures of the corresponding patients (53). Impact of EBV persistent infection on Topo IIa activation has been also investigated. A study group concluded that the specific viral kinase EBV BGLF4 enhances the decatenation activity of topoisomerase II inducing pre-mature chromosome condensation (54). They also observed and reported a significantly elevated EBV-depended reactivation of condensation of cellular chromatin in host NPC cells was critical for explaining the EBV-mediated reorganization of nuclear architecture.

Anti-topoisomerase Strategies in HNCs

Since the last decade, novel anti-topoisomerase inhibition strategies have been developed. Concerning breast adenocarcinoma, anthracyclines act as critical inhibitors of Topo IIa aberrant expression, especially in gene amplified cases (55). Additionally, Topo IIa gene amplification or deletion modifies the response rates of patients suffering from solid malignancies, such as endometrial or pancreatic cancers and also correlates with poor prognosis (56, 57). Interestingly, a study group analyzing Topo IIa over-expression in patients with LSCC that receive radiotherapy, showed that aberrant expression of Topo IIa-due to its

hypermethylation - increases radio-resistance rates (58). For this reason, novel anti-Topo IIa agents including epipodophyllotoxin-based F14512 drug and cisplatin induce radio-sensitivity in sub-groups of LSCC patients (59). The role of anti-Topo IIa drugs combined or not with radiotherapy is under investigation in HPV-depended cases. Recently, a clinical-molecular study analyzed the efficacy of pemetrexed and etoposide combined with cisplatin as therapeutic regimens in HPV positive patients with oral and oropharyngeal cancer. They observed that in Topo IIa and thymidylate synthase (TS) over-expressed cases these regimens offer increased response rates, although extended clinical trials are necessary for evaluating their benefits in patients characterized by specific molecular signatures (60). Concerning Topo I in LSSC and OSCCs, camptothecin, irinotecan, SN-38 etoposide, and teniposide are important inhibitors. Especially, a study group concluded that SN-38 is highly cytotoxic to OSCC cell lines (61). In combination with previously reported data regarding teniposide, another clinical-molecular study showed that the agent could significantly induce apoptotic and Topo IIa & I activity in OSCC, and inhibits cell growth (62).

Critically, mutations in *Topo I* gene negatively affect the response rates to the targeted therapeutic regimens mentioned above. A study group analyzing the Topo I mutational landscape observed that a single amino acid substitution (E418K) is responsible for increased resistance to camptothecin in NPC cell line cultures (63). Interestingly, in hematological malignancies Topo I affects camptothecin efficiency by inducing cleavage of specific tandem repeats (64). Additionally, mechanisms of anti-Topo I camptothecin-based therapy include decreased expression of O(6)-methylguanine-DNA methyltransferase (MGMT), an important enzyme involved in DNA stability and repair procedure, regulating also cytotoxicity in NPC cell line cultures treated by camptothecin (65). Concerning NPC, targeted therapy with combination of agents seems to provide a better result than monotherapy. In an experimental study, the synergistic activity of topotecan and chronomodulated radiation was analyzed on xenografted NPC tumors. The study group reported a significant enhancement of radiosensitivity in NPC cells (66). In addition to the previous mentioned anti-Topo I & IIa targeted strategies, there is a variety of experimentally analyzed agents, such as voreloxin, mansonone F (MF) derivatives, providing important results for future therapeutic protocols (67, 68). Furthermore, in modern anti-Topo regimens, the combination of two agents, such as docetaxel and topotecan, is experimentally enriched with other inhibitors, including granulocyte-colony-stimulating factor (G-CSF) or dovitinib - an orally bioavailable molecule which triggers a G_2/M arrest and interrupts receptor tyrosine kinases mediated signal transduction to nucleus (69, 70).

Conclusion

In conclusion, topoisomerases are significant molecules that regulate DNA structural and functional stability and homeostasis. A variety of HNC histopathological entities including LSCC, OSCC, and NPC demonstrate Topo I and IIa gene deregulation leading to an aberrant over-expression of these molecules. Novel anti-topoisomerase targeted strategies are crucial for preventing their abnormal activity and regulating the corresponding negative genetic events that drive malignancies to more aggressive phenotypes.

Conflicts of Interest

The Authors declare no conflicts of interest associated with this article.

Authors' Contributions

ET, EK, VR, VP, AC searched the literature and drafted the article, with ET being the major contributor in writing the article. DP, NM, DS, PP, AN collected the data provided by the corresponding references. All Authors read and approved the final article.

References

1 Albertson DG, Collins C, McCormick F and Gray JW: Chromosome aberrations in solid tumors. Nat Genet *34(4)*: 369-376, 2003. PMID: 12923544. DOI: 10.1038/ng1215

2 Salk JJ, Fox EJ and Loeb LA: Mutational heterogeneity in human cancers: origin and consequences. Annu Rev Pathol *5*: 51-75, 2010. PMID: 19743960. DOI: 10.1146/annurev-pathol-121808-102113

3 Polyak K, Haviv I and Campbell IG: Co-evolution of tumor cells and their microenvironment. Trends Genet *25(1)*: 30-38, 2009. PMID: 19054589. DOI: 10.1016/j.tig.2008.10.012

4 Loeb LA and Harris CC: Advances in chemical carcinogenesis: a historical review and prospective. Cancer Res *68(17)*: 6863-6872, 2008. PMID: 18757397. DOI: 10.1158/0008-5472.CAN-08-2852

5 Hanahan D and Weinberg RA: Hallmarks of cancer: the next generation. Cell *144(5)*: 646-674, 2011. PMID: 21376230. DOI: 10.1016/j.cell.2011.02.013

6 Stratton MR, Campbell PJ and Futreal PA: The cancer genome. Nature *458(7239)*: 719-724, 2009. PMID: 19360079. DOI: 10.1038/nature07943

7 Nicholson RI, Gee JM and Harper ME: EGFR and cancer prognosis. Eur J Cancer *37 Suppl 4*: S9-15, 2001. PMID: 11597399. DOI: 10.1016/s0959-8049(01)00231-3

8 Karachaliou N, Rosell R, Molina MA and Viteri S: Predicting resistance by selection of signaling pathways. Transl Lung Cancer Res *3(2)*: 107-115, 2014. PMID: 25806289. DOI: 10.3978/j.issn.2218-6751.2014.02.04

9 Wang JC: Cellular roles of DNA topoisomerases: a molecular perspective. Nat Rev Mol Cell Biol *3(6)*: 430-440, 2002. PMID: 12042765. DOI: 10.1038/nrm831

10 Champoux JJ: DNA topoisomerases: structure, function, and mechanism. Annu Rev Biochem *70*: 369-413, 2001. PMID: 11395412. DOI: 10.1146/annurev.biochem.70.1.369
11 Nagaraja V, Godbole AA, Henderson SR and Maxwell A: DNA topoisomerase I and DNA gyrase as targets for TB therapy. Drug Discov Today *22(3)*: 510-518, 2017. PMID: 27856347. DOI: 10.1016/j.drudis.2016.11.006
12 Ferrandina G, Petrillo M, Carbone A, Zannoni G, Martinelli E, Prisco M, Pignata S, Breda E, Savarese A and Scambia G: Prognostic role of topoisomerase-IIalpha in advanced ovarian cancer patients. Br J Cancer *98(12)*: 1910-1915, 2008. PMID: 18506140. DOI: 10.1038/sj.bjc.6604410
13 Cuylen S, Blaukopf C, Politi AZ, Müller-Reichert T, Neumann B, Poser I, Ellenberg J, Hyman AA and Gerlich DW: Ki-67 acts as a biological surfactant to disperse mitotic chromosomes. Nature *535(7611)*: 308-312, 2016. PMID: 27362226. DOI: 10.1038/nature18610
14 Heck MM and Earnshaw WC: Topoisomerase II: A specific marker for cell proliferation. J Cell Biol *103(6 Pt 2)*: 2569-2581, 1986. PMID: 3025219. DOI: 10.1083/jcb.103.6.2569
15 Niotis A, Tsiambas E, Fotiades PP, Ragos V and Polymeneas G: ki-67 and Topoisomerase IIa proliferation markers in colon adenocarcinoma. J BUON *23(7)*: 24-27, 2018. PMID: 30722108.
16 Reimers MS, Zeestraten EC, van Alphen TC, Dekker JW, Putter H, Saadatmand S, Liefers GJ, van de Velde CJ and Kuppen PJ: Combined analysis of biomarkers of proliferation and apoptosis in colon cancer: an immunohistochemistry-based study using tissue microarray. Int J Colorectal Dis *29(9)*: 1043-1052, 2014. PMID: 24950792. DOI: 10.1007/s00384-014-1930-y
17 Faratzis G, Tsiambas E, Rapidis AD, Machaira A, Xiromeritis K and Patsouris E: VEGF and ki 67 expression in squamous cell carcinoma of the tongue: An immunohistochemical and computerized image analysis study. Oral Oncol *45(7)*: 584-588, 2009. PMID: 18804402. DOI: 10.1016/j.oraloncology.2008.08.002
18 Stromar IK and Jakic-Razumovic J: The value of immunohistochemical determination of topoisomerase IIα and Ki67 as markers of cell proliferation and malignant transformation in colonic mucosa. Appl Immunohistochem Mol Morphol *22(7)*: 524-529, 2014. PMID: 24185119. DOI: 10.1097/PAI.0b013e3182a1c9c6
19 Huang SN, Williams JS, Arana ME, Kunkel TA and Pommier Y: Topoisomerase I-mediated cleavage at unrepaired ribonucleotides generates DNA double-strand breaks. EMBO J *36(3)*: 361-373, 2017. PMID: 27932446. DOI: 10.15252/embj.201592426
20 Schoeffler AJ and Berger JM: DNA topoisomerases: harnessing and constraining energy to govern chromosome topology. Q Rev Biophys *41(1)*: 41-101, 2008. PMID: 18755053. DOI: 10.1017/S003358350800468X
21 Lee JH and Berger JM: Cell cycle-dependent control and roles of DNA topoisomerase II. Genes (Basel) *10(11)*: 859, 2019. PMID: 31671531. DOI: 10.3390/genes10110859
22 Lyu YL, Lin CP, Azarova AM, Cai L, Wang JC and Liu LF: Role of topoisomerase IIbeta in the expression of developmentally regulated genes. Mol Cell Biol *26(21)*: 7929-7941, 2006. PMID: 16923961. DOI: 10.1128/MCB.00617-06
23 Vos SM, Tretter EM, Schmidt BH and Berger JM: All tangled up: how cells direct, manage and exploit topoisomerase function. Nat Rev Mol Cell Biol *12(12)*: 827-841, 2011. PMID: 22108601. DOI: 10.1038/nrm3228
24 Postow L, Crisona NJ, Peter BJ, Hardy CD and Cozzarelli NR: Topological challenges to DNA replication: conformations at the fork. Proc Natl Acad Sci U S A *98(15)*: 8219-8226, 2001. PMID: 11459956. DOI: 10.1073/pnas.111006998
25 Orlando L, Del Curto B, Gandini S, Ghisini R, Pietri E, Torrisi R, Balduzzi A, Cardillo A, Dellapasqua S, Veronesi P, Viale G, Goldhirsch A and Colleoni M: Topoisomerase IIalpha gene status and prediction of pathological complete remission after anthracycline-based neoadjuvant chemotherapy in endocrine non-responsive Her2/neu-positive breast cancer. Breast *17(5)*: 506-511, 2008. PMID: 18456496. DOI: 10.1016/j.breast.2008.03.007
26 Surowiak P, Materna V, Kaplenko I, Spaczynski M, Dietel M, Lage H and Zabel M: Topoisomerase 1A, HER/2neu and Ki67 expression in paired primary and relapse ovarian cancer tissue samples. Histol Histopathol *21(7)*: 713-720, 2006. PMID: 16598670. DOI: 10.14670/HH-21.713
27 Chen GL, Tian YQ, Wu JL, Li N and Guo MQ: Antiproliferative activities of Amaryllidaceae alkaloids from Lycoris radiata targeting DNA topoisomerase I. Sci Rep *6*: 38284, 2016. PMID: 27922057. DOI: 10.1038/srep38284
28 Lu B, Zhang H, Zhang T, Cai Y, Hu Y, Zheng H and Li B: Topoisomerase I expression is associated with prognosis in postoperative non-small cell lung cancer patients. Thorac Cancer *7(4)*: 486-494, 2016. PMID: 27385993. DOI: 10.1111/1759-7714.12359
29 Smith DH, Christensen IJ, Jensen NF, Markussen B, Rømer MU, Nygård SB, Müller S, Nielsen HJ, Brünner N and Nielsen KV: Mechanisms of topoisomerase I (TOP1) gene copy number increase in a stage III colorectal cancer patient cohort. PLoS One *8(4)*: e60613, 2013. PMID: 23577133. DOI: 10.1371/journal.pone.0060613
30 Gilbert D, Chalmers A and El-khamisy S: Topoisomerase I inhibition in colorectal cancer: biomarkers and therapeutic targets. British Journal of Cancer *106(1)*: 18-24, 2019. DOI: 10.1038/bjc.2011.498
31 Ali J, Sabiha B, Jan HU, Haider SA, Khan AA and Ali SS: Genetic etiology of oral cancer. Oral Oncol *70*: 23-28, 2017. PMID: 28622887. DOI: 10.1016/j.oraloncology.2017.05.004
32 Verma G, Vishnoi K, Tyagi A, Jadli M, Singh T, Goel A, Sharma A, Agarwal K, Prasad SC, Pandey D, Sharma S, Mehrotra R, Singh SM and Bharti AC: Characterization of key transcription factors as molecular signatures of HPV-positive and HPV-negative oral cancers. Cancer Med *6(3)*: 591-604, 2017. PMID: 28155253. DOI: 10.1002/cam4.983
33 Mattila R, Rautava J and Syrjänen S: Human papillomavirus in oral atrophic lichen planus lesions. Oral Oncol *48(10)*: 980-984, 2012. PMID: 22658678. DOI: 10.1016/j.oraloncology.2012.04.009
34 Mattila R, Alanen K and Syrjänen S: Immunohistochemical study on topoisomerase IIalpha, Ki-67 and cytokeratin-19 in oral lichen planus lesions. Arch Dermatol Res *298(8)*: 381-388, 2007. PMID: 17072627. DOI: 10.1007/s00403-006-0711-z
35 Hafian H, Venteo L, Sukhanova A, Nabiev I, Lefevre B and Pluot M: Immunohistochemical study of DNA topoisomerase I, DNA topoisomerase II alpha, p53, and Ki-67 in oral preneoplastic lesions and oral squamous cell carcinomas. Hum Pathol *35(6)*: 745-751, 2004. PMID: 15188142. DOI: 10.1016/j.humpath.2004.02.004
36 Oliveira-Costa JP, Oliveira LR, da Silveira GG, Soave DF, Soares FA and Ribeiro-Silva A: Topoisomerase expression in

oral squamous cell carcinoma: relationship with cancer stem cells profiles and lymph node metastasis. J Oral Pathol Med *41(10)*: 762-768, 2012. PMID: 22672768. DOI: 10.1111/j.1600-0714.2012.01174.x

37 Segawa E, Sakurai K, Kishimoto H, Takaoka K, Noguchi K, Hashitani S, Hirota S and Urade M: Expression of cyclooxygenase-2 and DNA topoisomerase II alpha in precancerous and cancerous lesions of the oral mucosa. Oral Oncol *44(7)*: 664-671, 2008. PMID: 17996482. DOI: 10.1016/j.oraloncology.2007.08.014

38 Siegel RL, Miller KD and Jemal A: Cancer statistics, 2019. CA Cancer J Clin *69(1)*: 7-34, 2019. PMID: 30620402. DOI: 10.3322/caac.21551

39 Morbini P and Benazzo M: Papillomavirus umano e carcinomi del tratto aerodigestivo: il punto sulle evidenze nella babele dei dati scientifici. Acta Otorhinolaryngologica Italica *36(4)*: 249-258, 2021. DOI: 10.14639/0392-100X-853

40 Malm IJ, Rooper LM, Bishop JA, Ozgursoy SK, Hillel AT, Akst LM and Best SR: Molecular and immunologic analysis of laryngeal squamous cell carcinoma in smokers and non-smokers. Am J Otolaryngol *40(2)*: 213-217, 2019. PMID: 30553600. DOI: 10.1016/j.amjoto.2018.11.009

41 Wittekindt C, Sittel C, Kvasnicka HM and Eckel HE: Immunohistochemistry of whole-organ sections of advanced human laryngeal cancer. Eur Arch Otorhinolaryngol *263(8)*: 741-746, 2006. PMID: 16683119. DOI: 10.1007/s00405-006-0055-5

42 Cuylen S, Blaukopf C, Politi AZ, Müller-Reichert T, Neumann B, Poser I, Ellenberg J, Hyman AA and Gerlich DW: Ki-67 acts as a biological surfactant to disperse mitotic chromosomes. Nature *535(7611)*: 308-312, 2016. PMID: 27362226. DOI: 10.1038/nature18610

43 Papanikolaou V, Chrysovergis A, Mastronikolis N, Tsiambas E, Ragos V, Peschos D, Stavraka C, Roukas D and Kyrodimos E: Topoisomerase IIa protein expression patterns in laryngeal squamous cell carcinoma. Anticancer Res *40(2)*: 807-811, 2020. PMID: 32014923. DOI: 10.21873/anticanres.14012

44 Horibe Y, Murakami M, Komori K, Imaeda Y and Kasahara M: Expression of topoisomerase II alpha, Ki-67 and p53 in early stage laryngeal carcinomas not featuring vocal cord fixation. APMIS *108(10)*: 689-696, 2000. PMID: 11200824. DOI: 10.1034/j.1600-0463.2000.d01-16.x

45 Feng Y, Zhang H, Gao W, Wen S, Huangfu H, Sun R, Bai W and Wang B: Expression of DNA topoisomerase II-α: Clinical significance in laryngeal carcinoma. Oncol Lett *8(4)*: 1575-1580, 2014. PMID: 25202370. DOI: 10.3892/ol.2014.2367

46 Shvero J, Koren R, Shvili I, Yaniv E, Sadov R and 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 *130(6)*: 934-939, 2008. PMID: 19019771. DOI: 10.1309/AJCPROG61USKCBEI

47 Durand-Faucher K, Rabinovitch-Chable H, Dzugan H, Charret S, Aubry K, Genet D, Léobon S, Tubiana-Mathieu N, Cook-Moreau J, Rigaud M and Sturtz FG: A quantitative RT-PCR method to determine topoisomerase I mRNA levels in human tissue samples. Clin Chem Lab Med *43(7)*: 707-714, 2005. PMID: 16207129. DOI: 10.1515/CCLM.2005.120

48 Hanagiri T, Ono K, Kuwata T, Takenaka M, Oka S, Chikaishi Y, Shigematsu Y, Nagata Y, Shimokawa H, Nakagawa M, Uramoto H, So T and Tanaka F: Evaluation of topoisomerase I/topoisomerase IIalpha status in esophageal cancer. J UOEH *33(3)*: 205-216, 2011. PMID: 21913377. DOI: 10.7888/juoeh.33.205

49 Bei JX, Li Y, Jia WH, Feng BJ, Zhou G, Chen LZ, Feng QS, Low HQ, Zhang H, He F, Tai ES, Kang T, Liu ET, Liu J and Zeng YX: A genome-wide association study of nasopharyngeal carcinoma identifies three new susceptibility loci. Nat Genet *42(7)*: 599-603, 2010. PMID: 20512145. DOI: 10.1038/ng.601

50 Tsiambas E, Mastronikolis NS, Fotiades PP, Ragos V, Kavantzas N and Lazaris AC: Epstein-Barr virus microRNAs in nasopharyngeal carcinoma. Pathol Oncol Res *26(2)*: 1347-1348, 2020. PMID: 30852739. DOI: 10.1007/s12253-019-00631-2

51 Lan J, Huang HY, Lee SW, Chen TJ, Tai HC, Hsu HP, Chang KY and Li CF: TOP2A overexpression as a poor prognostic factor in patients with nasopharyngeal carcinoma. Tumour Biol *35(1)*: 179-187, 2014. PMID: 23897556. DOI: 10.1007/s13277-013-1022-6

52 Chen F, Shen C, Wang X, Wang H, Liu Y, Yu C, Lv J, He J and Wen Z: Identification of genes and pathways in nasopharyngeal carcinoma by bioinformatics analysis. Oncotarget *8(38)*: 63738-63749, 2017. PMID: 28969025. DOI: 10.18632/oncotarget.19478

53 Lee CP, Chen JY, Wang JT, Kimura K, Takemoto A, Lu CC and Chen MR: Epstein-Barr virus BGLF4 kinase induces premature chromosome condensation through activation of condensin and topoisomerase II. J Virol *81(10)*: 5166-5180, 2007. PMID: 17360754. DOI: 10.1128/JVI.00120-07

54 Schindlbeck C, Mayr D, Olivier C, Rack B, Engelstaedter V, Jueckstock J, Jenderek C, Andergassen U, Jeschke U and Friese K: Topoisomerase IIalpha expression rather than gene amplification predicts responsiveness of adjuvant anthracycline-based chemotherapy in women with primary breast cancer. J Cancer Res Clin Oncol *136(7)*: 1029-1037, 2010. PMID: 20052594. DOI: 10.1007/s00432-009-0748-4

55 Tsiambas E, Alexopoulou D, Lambropoulou S, Gerontopoulos K, Karakitsos P and 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 *16(3)*: 1424-1431, 2006. PMID: 16803541. DOI: 10.1111/j.1525-1438.2006.00562.x

56 Tsiambas E, Karameris A, Tiniakos DG and Karakitsos P: Evaluation of topoisomerase IIa expression in pancreatic ductal adenocarcinoma: a pilot study using chromogenic in situ hybridization and immunohistochemistry on tissue microarrays. Pancreatology *7(1)*: 45-52, 2007. PMID: 17449965. DOI: 10.1159/000101877

57 Kim JS, Kim SY, Lee M, Kim SH, Kim SM and 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 *16(4)*: 558-566, 2015. PMID: 25719218. DOI: 10.1080/15384047.2015.1017154

58 Mouawad F, Gros A, Rysman B, Bal-Mahieu C, Bertheau C, Horn S, Sarrazin T, Lartigau E, Chevalier D, Bailly C, Lansiaux A and Meignan S: The antitumor drug F14512 enhances cisplatin and ionizing radiation effects in head and neck squamous carcinoma cell lines. Oral Oncol *50(2)*: 113-119, 2014. PMID: 24290982. DOI: 10.1016/j.oraloncology.2013.11.003

59 Kim YR, Lee B, Byun MR, Lee JK and Choi JW: Evaluation of pemetrexed and etoposide as therapeutic regimens for human papillomavirus-positive oral and oropharyngeal cancer. PLoS One *13(7)*: e0200509, 2018. PMID: 29995943. DOI: 10.1371/journal.pone.0200509

60 Tamura N, Hirano K, Kishino K, Hashimoto K, Amano O, Shimada J and Sakagami H: Analysis of type of cell death

induced by topoisomerase inhibitor SN-38 in human oral squamous cell carcinoma cell lines. Anticancer Res *32(11)*: 4823-4832, 2012. PMID: 23155248.

61 Li J, Chen W, Zhang P and Li N: Topoisomerase II trapping agent teniposide induces apoptosis and G2/M or S phase arrest of oral squamous cell carcinoma. World J Surg Oncol *4*: 41, 2006. PMID: 16822322. DOI: 10.1186/1477-7819-4-41

62 Chang JY, Liu JF, Juang SH, Liu TW and Chen LT: Novel mutation of topoisomerase I in rendering cells resistant to camptothecin. Cancer Res *62(13)*: 3716-3721, 2002. PMID: 12097280.

63 Kjeldsen E, Nielsen CJF, Roy A, Tesauro C, Jakobsen AK, Stougaard M and Knudsen BR: Characterization of camptothecin-induced genomic changes in the camptothecin-resistant T-ALL-derived cell line CPT-K5. Cancer Genomics Proteomics *15(2)*: 91-114, 2018. PMID: 29496689. DOI: 10.21873/cgp.20068

64 Kuo CC, Liu JF and Chang JY: DNA repair enzyme, O6-methylguanine DNA methyltransferase, modulates cytotoxicity of camptothecin-derived topoisomerase I inhibitors. J Pharmacol Exp Ther *316(2)*: 946-954, 2006. PMID: 16258022. DOI: 10.1124/jpet.105.095919

65 Zhang Y, Chen X, Ren P, Su Z, Cao H, Zhou J, Zou X, Fu S, Lin S, Fan J, Yang B, Sun X, Zhou Y, Chen Y, Yang L and Wu J: Synergistic effect of combination topotecan and chronomodulated radiation therapy on xenografted human nasopharyngeal carcinoma. Int J Radiat Oncol Biol Phys *87(2)*: 356-362, 2013. PMID: 23906928. DOI: 10.1016/j.ijrobp.2013.05.047

66 Hoch U, Lynch J, Sato Y, Kashimoto S, Kajikawa F, Furutani Y and Silverman JA: Voreloxin, formerly SNS-595, has potent activity against a broad panel of cancer cell lines and in vivo tumor models. Cancer Chemother Pharmacol *64(1)*: 53-65, 2009. PMID: 18931998. DOI: 10.1007/s00280-008-0850-3

67 Wu WB, Ou JB, Huang ZH, Chen SB, Ou TM, Tan JH, Li D, Shen LL, Huang SL, Gu LQ and Huang ZS: Synthesis and evaluation of mansonone F derivatives as topoisomerase inhibitors. Eur J Med Chem *46(8)*: 3339-3347, 2011. PMID: 21600681. DOI: 10.1016/j.ejmech.2011.04.059

68 Tsao AS, Shin DM, Palmer JL, Lee JS and Glisson BS: Phase I evaluation of docetaxel and topotecan for patients with advanced solid tumors. Cancer *100(10)*: 2240-2245, 2004. PMID: 15139070. DOI: 10.1002/cncr.20238

69 Man WY, Mak JP and Poon RY: Dovitinib induces mitotic defects and activates the G2 DNA damage checkpoint. J Cell Mol Med *18(1)*: 143-155, 2014. PMID: 24238094. DOI: 10.1111/jcmm.12176

70 Wang G, Huang H, Gao J, Chen P, You W, Wu B and Wang M: Tissue microarray analysis of topoisomerase IIalpha protein in gastric adenocarcinomas: histogenetic and prognostic implications. Cancer Genomics Proteomics *8(3)*: 127-133, 2011. PMID: 21518818.

Received March 18, 2021
Revised May 6, 2021
Accepted May 7, 2021