JBUON 2018; 23 (Suppl 1): S24-S27
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

SPECIAL MOLECULAR REVIEW ARTICLE

ki-67 and Topoisomerase IIa proliferation markers in colon adenocarcinoma

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Summary

Aberrant cell proliferation is a major cause in the development and progression of carcinogenic process. Epithelia characterized by increased mitotic rates accumulate easily gross numerical and structural chromosomes (polysomy/aneuploidy) and specific gene (deletions, amplifications, point mutations, translocations) deregulations that lead to their progressive neoplastic and finally malignant transformation. Molecules that are critical for evaluating the proliferation status of the corresponding tissues include mainly ki-67 (cytogenetic band: 10q26.2), and also Topoisomerase IIa/Topo IIa (cytogenetic band: 17q21.2). Both of them demonstrate different expression patterns in every cell cycle phase and their estimated expression as Nuclear Labeling Index (NLI) is a very useful tool for assessing the aggressiveness of the examined pre- and malignant tissues. In fact, ki-67 expression increases as a cell progresses through the cell cycle, with highest expression being seen in G2/M phase cell, whereas Topo IIa is expressed in proliferating cells in the late S phase with a peak in G2-M phases. Concerning colon adenocarcinoma, high expression levels of them seem to correlate with advanced disease and also with modified response rates to specific chemotherapeutic agents, such as doxorubicin, an inhibitor of Topo IIa. In the current molecular review we explored the role of these proliferative markers in colon adenocarcinoma and their influence in the tumor biological behavior.

Key words: *cell cycle, colon carcinoma, ki-67, proliferation, topoisomerase*

Introduction

Intra-cellular genomic instability includes a variety of numerical and structural chromosome (polysomy/aneuploidy) and specific gene (deletions, amplifications, point mutations, translocations) deregulations. Extensive molecular analyses have shown that the hallmarks of cancer are referred predominantly to deregulated procedures inside the cell and also inside the tumor microenvironment. A complex of genetic and metabolic reactions, such as resisting cell death, sustaining proliferative signaling, evading growth suppressors, inducing angiogenesis, enabling replicative immortality, and activating invasion/metastasis, combined with avoiding immune destruction and deregulating cellular epigenetics characterize the multistep and multifunctional cell malignant transformation and tumor rise [1]. Concerning colon adenocarcinoma (CA), it seems that two main mechanisms of genetic deregulation are involved in the malignant transformation of normal colon glandular epithelia [2]. Gross chromosomal instability, as the result of chromosomal polysomy/aneuploidy,

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Received: 04/06/2018; Accepted: 01/07/2018

lead to CA in the majority of analyzed cases (~85%), whereas the rest demonstrate chromosomal stability (diploid predominantly patterns). A subset of the chromosomally unstable cases are characterized by microsatellite instability (MSI) due to MLH1 gene promoter CpG island hypermethylation and also to specific mutations in other DNA mismatch repair (MMR) genes, including MSH2 and MSH6 that are involved in the cases of hereditary non-polyposis colorectal cancers (HNPCC) [3,4]. Finally, a small fraction of patients with CA is characterized by a complex genetic signature and molecular heterogeneity due to a combination of these mechanisms [5]. Undoubtedly, cancer cell proliferation rate is a significant factor for evaluating the aggressiveness of the examined malignant tissues. NLI is a very useful tool in estimating and measuring the proliferative (mitotic) index in cancer cells based on some critical molecules nuclear expression levels. Among them, ki-67 predominantly and also Topo IIa proteins provide important information regarding the intratumor activity. In the current molecular review we explored the significance of their expression patterns in CA progression and their influence in the tumor biological behavior.

ki-67 gene expression in CA

ki-67 gene located on chromosome 10 (cytogenetic band: 10q26.2) encodes a protein which is expressed in the nucleolus in all cell cycle phases except Go (arrest phase) [6]. In fact, ki-67 expression increases as a cell progresses through the cell cycle, with highest expression being seen in G2/M phase cells [7]. Concerning the nuclear microchromosomal environment, it is located on the surface of the mitotic chromosome, which is the perichromosomal layer [8]. Furthermore, the Ki-67 protein acts as a surfactant, dispersing chromosomes and enabling independent chromosome motility and also is partially implicated in chromatin organization [9,10]. The molecule is visible due to its immunohistochemical (IHC) expression within the nucleus in the interphase, whereas in mitosis most of the protein is relocated to the surface of the chromosomes. Monoclonal antibodies interact with the ki-67 antigen on its *in situ* position, demonstrating different levels of proliferative activity. ki-67 acts as a pure proliferative marker by measuring the positive tumor cells fraction known as NLI in every cellular localization during the cell-division cycle. ki-67 is frequently overexpressed in CA. Interestingly, the imbalance between proliferation and apoptosis rates seem to affect CA biological behavior. A study group analyzing stage I-IV CAs on tissue microarray (TMA) sections observed that combined apoptosis proliferation (CAP) index can be used as a prognostic marker in the corresponding patients, particularly in left-sided, microsatellite stable tumors with tumor-node-metastasis which was associated with worse prognosis [11]. Similarly, another study group explored the potential role of the combined ki-67 and carcinoembryonic antigen (CEA) preoperative expression in IIA stage CA patients. Their stratified analysis showed that elevated CEA serum level combined with high expression of ki-67 was associated with poorer prognosis (3-year DFS 70 %) [12]. Approaching the marker in the field of pre-malignant lesions that progressively are transformed to cancerous epithelia, a study group investigated the correlation between ki-67 and vitamin D serum levels in conjuction with b-catenin expression in the corresponding tissues. They concluded that high ki-67 expression NLIs were correlated with insufficient vitamin D concentrations and also low to negative nuclear β-catenin expression [13]. Concerning ki-67 expression in pre-malignant neoplastic serrated lesions that are classified as hyperplastic polyps (HPs), sessile serrated adenomas/polyps (SSA/Ps), and traditional serrated adenomas (TSAs) according to their morphology, another study group recently showed that HPs demonstrated the highest levels, whereas $p16I^{NK4a}$ suppressor gene was downregulated [14]. This reflects an early-stage activated proliferation mechanism in these lesions.

Topoisomerase IIa gene expression in CA

Topoisomerases represent a class of nucleic enzymes, which affect the topological structure of DNA. The main members of the family are Topoisomerase I (gene location 20q11), Topoisomerase II alpha (gene location 17q21) and Topoisomerase IIb (gene location 3p24). Topo IIa and b isomers' combined action promotes temporarily cutting and rejoining the DNA double helix. 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 mass of 170 kDa, 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 [15]. Correlating Ki-67 to Topo IIa duration of expression, Topo IIa protein level seems to provide a better estimation of the number of actively proliferating cells and for this reason it could be used as a reliable marker of proliferation (Figure 1). Furthermore, topoisomerases' inhibition promotes cell death and for this reason they are targets

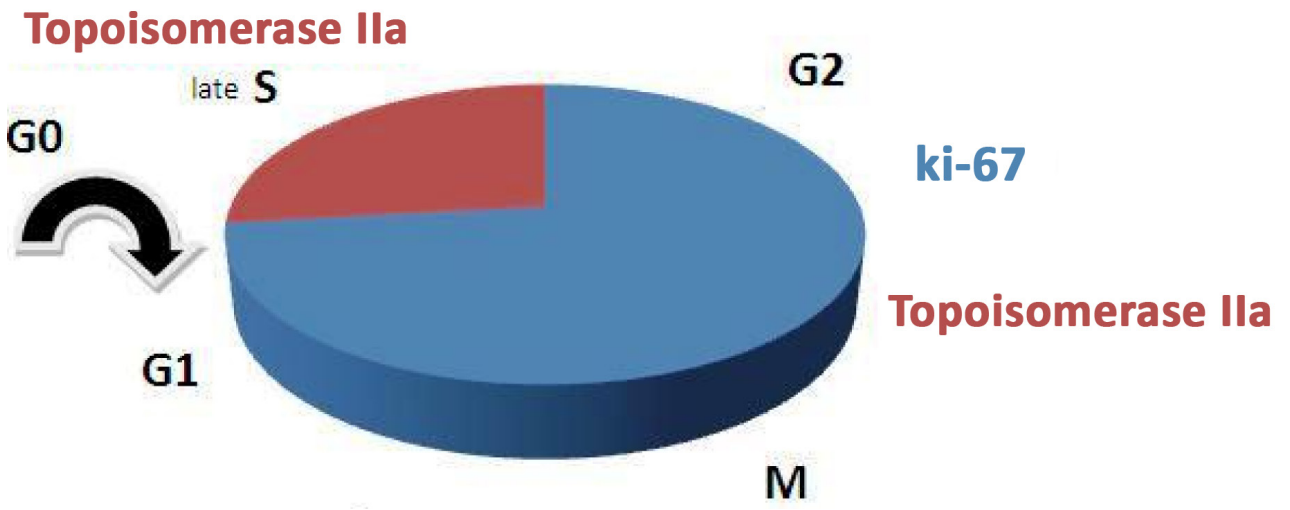

Figure 1. Schematic representation of ki-67 and Topoisomerase IIa expression in cell cycle phases. ki-67 demonstrates a progressively over expression in G1/S/G2/M phases, whereas Topoisomerase IIa in late S phase with a peak in G2/M phases. For this reason the molecule is considered as a more specific proliferative marker focusing on DNA replication compared to ki-67.

for specific chemotherapy. Some clinical studies have shown that adjuvant chemotherapy based on a combination of anthracyclines (doxorubicin) with etoposide and fluorouracil/cyclophosphamide or carboplatin/paclitaxel is very effective in patients with breast, endometrial or also ovarian cancer that demonstrate aberrant protein expression due to gene amplification predominantly [16,17]. Concerning CA, Topo IIa immunohistochemical (IHC) overexpression seems to be correlated with CA recurrences by comparison to their primary locations [18]. This observation enhances the opinion that Topo IIa should be considered as one of the markers of drug resistance in relapsing colon cancer. Additionally, a subset of CA patients demonstrate a specific gene signature based on a multi-amplification of two or more oncogenes such as c-myc, EGFR, cyclin D1, HER2 combined with Topo IIa. It should be mentioned that HER2 and Topo IIa share a common genetic band on chromosome 17 (17q) which reflects their combined deregulation due to gene amplification in a fraction of patients. A study group analyzed a significant series of CA tissue by implementing fluorescence *in situ* hybridization (FISH) and concluded that gene amplified cases leading to Topo IIa protein over expression should be targets for specific chemotherapy by applying anthracyclines [19]. Furthermore, a multi-targeted therapeutic regimen (monoclonal antibodies) should be applied in a sub-set of them based on the molecular criterion of multi-gene amplifications, especially in HER2/EGFR/ Topo IIa amplified genotypes.

In conclusion, ki-67, as a traditional proliferation marker and also Topo IIa as a significant protein are useful agents for evaluating deregulated cell cycle metabolism due to their NLI expressions. Because of its relation with chemotherapeutic agents (anthracyclines) that inhibit its activity and for its ability to be detected by IHC expression in S phase -besides G2/M- , Topo IIa is a critical molecule in the management of carcinomas of different histo-genetic origin, as it happens in a subset of patients with CA. Finally, both of the proliferative markers are correlated with early stage mucosal dysplasia determination (moderate or high expression in adenomas and also inflammatory bowel disease) [20]..

Conflict of interests

The authors declare no conflict of interests.

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