RESEARCH ARTICLE

The Effect of Galectin-3 on Apoptosis

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***Abstract*:** Cancer is a disease caused by changes in the critical genes that control cell proliferation, differentiation, survival and apoptosis. Apoptotic cell death is an important mechanism and target for the anti-cancer treatment. A characteristic finding in many types of cancer is a reduction in apoptosis. Galectin-3 (Gal-3) is a pleiotropic lectin that plays an important role in cell proliferation, adhesion, differentiation, angiogenesis, and apoptosis. Therefore, synthetic galectin-3 inhibitors are of utmost importance for development of new antitumor therapeutic strategies. Galectin-3 is mainly found in the cytoplasm, also seen in the nucleus and can be secreted by non-classical, secretory pathways. In general, secreted galectin-3 mediates cell migration, cell adhesion and cell–cell interactions through the binding with high affinity to galactose-containing glycoproteins on the cell surface. Cytoplasmic galectin-3 exhibits anti-apoptotic activity and regulates several signal transduction pathways, whereas nuclear galectin-3 has been associated with premRNA splicing and gene expression. During the past decade, extensive progress has been made toward understanding the molecular basis for the regulation of apoptosis. In this review, we have focused on the role of galectin-3 in tumor metastasis with special emphasis on apoptosis.

***Keywords*:** Galectin-3; Apoptosis; BCL-2; Survivin

1. Introduction

Cancer is a disease caused by changes in the critical genes that control cell proliferation, differentiation, survival and apoptosis[1]. Apoptotic cell death is an important mechanism and target for the anti-cancer treatment. Cancer spreads by penetrating the blood vessels and the lymphatic system. The physiological cell death that occurs in multi-cellular organisms is called “programmed cell death” or apoptosis and involves a complex network of biochemical pathways that normally ensure a homeostatic balance between cellular proliferation and turnover in nearly all tissues. Apoptosis is essential for the body, as its disregulation can lead to several diseases including cancer. During tumor progression cancer cells can develop ingenious mechanisms to escape the immune system most notably an increased resistance to apoptosis[2].

Oxidative damage has been attributed to the regulation of apoptotic genes. These mechanisms play key roles for the pathogenesis of proliferation and cell death[3,4]. Nitric oxide synthase (NOS) is responsible for the conversion of L-arginine to L-citruline to produce nitric oxide (NO). There are three isoforms of NOS, namely, endothelial NOS (eNOS; NOS1), inducible NOS (iNOS; NOS2), and neural NOS (nNOS; NOS3). Reactive oxygen species (ROS) are very important to produce necrotic or apoptotic cell death in many types of cancer[5]. Inhibition of apoptosis has been shown to inhibit nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathway[6]. These reactive molecules also contribute to oxidative damage of membrane lipids and damage to DNA which causes apoptosis by Bcl-2 and caspase activation[7]. Apoptosis encompasses many processes which normally occurs during development but is also useful to eliminate carcinogenic cells[8].

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Apoptosis, or programmed cell death, is the process whereby harmful, old or unwanted cells are killed. A characteristic finding in many types of cancer is a reduction in apoptosis[9]. There are couples of proteins that are related to apoptosis. The release of cytochrome c from mitochondria to cytosol is activated during mitochondria-mediated apoptosis. Cytochrome c, following its release to cytosol, cytosolic apoptotic protease activating factor 1 (Apaf-1) and procaspase-9 assemble to form apoptosom[10]. Procaspase-9 converts into caspase-9 in this complex and caspase 9 activates caspase-3 and-7 which cause cell death[11]. One of the most important mechanisms of apoptosis modulators is the Bcl-2 gene[12]. Anti-apoptotic effect achieves by preventing cytochrome c release and the activation of effector protease. Reduction in the levels of Bcl-2 cells leads to apoptosis, whereas an increase prevents cells from dying[13]. Nuclear Factor kappa B (NF-κB) is a protein which is believed to have an important role in angiogenic and apoptopic mechanisms in cancer[14].

Carbohydrate-protein interactions have been in evidence since they are involved in many biochemical and biological processes, such as downstream cell signaling, cell-cell and matrix- extracellular interactions, cell growth regulation, apoptosis and cancer metastasis[15]. During tumor development the alteration in cell survival and growth, as well as cell migration and antitumor immunity can be correlated with a diversity of glycosylation mechanisms on cell surface, leading to wide complex structures to be decoded by glycan-binding proteins (lectins)[16]. Thus, for deep understanding of what factors take place during the initial and late stages of neoplastic diseases it is crucial to consider the identification and expression of carbohydrate-binding proteins, such as galectins, as important targets for development of antitumor drugs[17,18]

Galectin-3 (Gal-3), a multifunctional protein of an expanding family of β-galactoside–binding animal lectins, is mainly produced by macrophages, and is implicated in a variety of biologic events, such as inflammation and angiogenesis[19]. Galectins are mainly found in the cytosol and the nucleus, and 14 members have been identified. Galectins are classified into three subgroups based on their structure, including: (a)prototype (galectin-1, -2, -5, -7, -10, -11, -13, and -14); (b)chimera type (galectin-3); and (c) tandem repeat type(galectin-6, -8, -9, and -12)[20]. Gal-3, formerly known as the Mac-2 antigen, is a chimeratype ～30-kDa carbohydrate-binding protein[21]. It is composed of a short NH2 terminal domain, which decides specific cellular targets; a repetitive collagen-like sequence, which serves as a substrate for matrix metalloproteinases; and a carboxyl terminal domain that contains the carbohydrate-binding region[22]. Gal-3 is also found in the cytoplasm and perinuclear mitochondrial membranes, where it is involved in the control of apoptosis, possibly through an interaction with the Bcl-2 protein[23]. In vitro experiments have shown that nuclear Gal-3 can bind nucleic acids[24]. Proliferating fibroblasts present with increased nuclear expression of Gal-3, compared to quiescent cells[25]. A large number of reports show that Gal-3 is mainly secreted by macrophages that mediate chronic and acute inflammation, innate and adaptive immunity, as well as surfactant homeostasis. Califice demonstrate, for the first time, that nuclear Gal-3 has a major negative impact on the malignant capacities of the cancer cells[25]. It is now well known that the biological behavior of each tumor depends on a number of factors, including the programmed cell death (apoptosis). Thus, abnormalities in apoptosis-regulating proteins can contribute to tumor progression and aggressiveness[26].

Cytoplasmic Gal-3 is associated with decreased susceptibility to apoptosis, while nuclear Gal-3 leads to increased induced apoptosis[25]. The latter observation is compatible with the recent report that leptomycin B increased the cisplatin-induced apoptosis of Gal-3-expressing BT-549 breast cancer cells[27]. This suggests a dual role for Gal-3 in apoptosis regulation, depending on its localization. The antiapoptotic activity of cytoplasmic Gal-3[28] could be mediated by the NWGR motif shared with Bcl-2, as shown by mutation[29]. Cytoplasmic Gal-3 abolished PARP cleavage, probably by inhibition of caspase activation. However, neither significant differences in caspase-8 and -9 cleavages nor in their specific activities were observed between LNCaP cells expressing cytoplasmic Gal-3 and controls. In BT-549 breast cancer cells, Gal-3 inhibits the intrinsic apoptotic pathway (but not the extrinsic pathway)[30]. However, Gal-3 counteracts TNF- induced apoptosis, involving the extrinsic pathway, in another breast cancer cell line[31]. In LNCaP cells, cytoplasmic Gal-3 inhibits both intrinsic (actinomycin D or X-ray irradiation) and extrinsic (TNF-a)

apoptotic pathways. Nuclear Gal-3 significantly increased PARP cleavage, caspase-8 activation and caspase-9 activity. Caspase-8 cleavage was not expected since actinomycin D should involve the caspase-8-independent intrinsic pathway. However, the intrinsic and extrinsic pathways appear interdependent in LNCaP cells[25].

It is now well known that the biological behavior of each tumor depends on a number of factors, including the programmed cell death (apoptosis). Thus, abnormalities in apoptosis-regulating proteins can contribute to tumor progression and aggressiveness. Members of the Bcl-2 family play an important role in the regulation of apoptotic pathways. Bcl-2 protein is an antiapoptotic molecule that maintains the integrity of the mitochondrial membrane and prevents cytochrome c release from mitochondria, needed for proteolytic degradation of the cell by caspases, which are the final executors of programmed cell death. Unlike Bcl-2, its homologue Bax allows disruption of mitochondrial membrane and caspases activation, thus being a protein with a pro-apoptotic function. It has been assumed that the ratio between Bcl-2 and Bax decides whether the cell will undergo apoptosis, that is, Bcl-2/Bax and Bax/Bax dimers are able to prolong cell life or induce cell death, respectively[26].

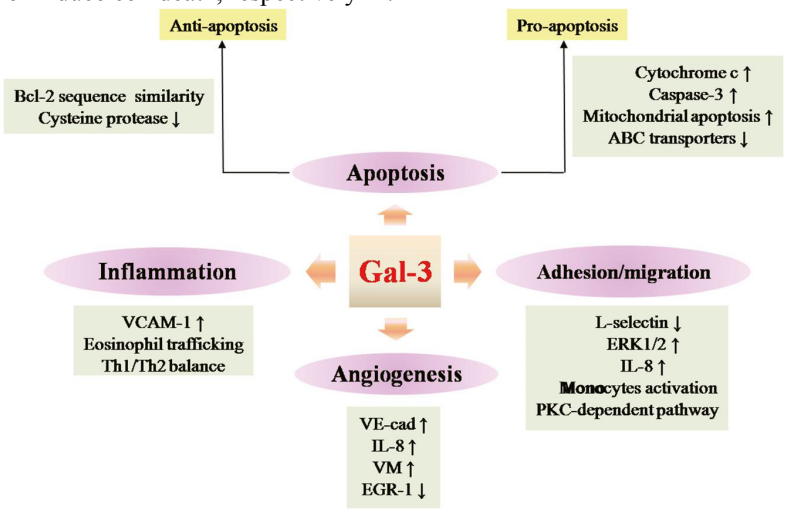

Figure 1; The role of Gal-3 in cell apoptosis, adhesion, migration, angiogenesis and inflammation[19].

Kawachi *et al*.[32] reported that primary lesions of PTC with metastasis contained a significantly higher concentration of galectin-3 than those without metastases, although the expression of galectin-3 was markedly decreased in metastatic compared with primary lesions. Taking into account the numerous functions of galectin-3 in cancer cell biology, including cell growth regulation, adhesion, angiogenesis, and apoptosis, it could be expected that it may play different roles in different tumor cell types and even in different stages of tumor progression in the same type of human tumor. With regards to the thyroid, the fact that galectin-3 gene is up-regulated in the early phase of malignant transformation and decreases through differentiation suggests that high galectin-3 levels are not unfavorable parameter for the thyroid oncology patients[26].

Another molecule influencing the apoptotic balance in cancer tissue was identified as survivin, a member of the inhibitor of apoptosis protein (IAP) family[33]. Survivin plays a role in regulating mitosis by localization to the mitotic spindle and interaction with tubulin, and also interplays with the apoptotic machinery downstream to mitochondria to inhibit caspase activation. Survivin is not detectable in normal adult tissues, but is prominently overexpressed in the most common human cancers[34]. Survivin, a member of the IAP family, has been shown to block apoptosis at the effector phase, that is, interplays with the apoptotic machinery downstream to mitochondria to inhibit caspase activation[35]. Overexpression of survivin has been found in a variety of human cancers including thyroid malignancies and associated with tumor aggressiveness and a poor prognosis[35].

Selemetjev *et al* demonstrated that high survivin expression strongly correlated with the presence of lymph node metastases in PTC and ATC patients. In addition, survivin expression was significantly higher in clinically advanced TNM stages (TNM stage III/IV) than in limited stages, which confirms its association with progression of thyroid tumor malignancy[26]. Interestingly, it has recently been shown that molecular interaction between survivin and XIAP (a

member of the IAP family) stimulates tumor cell invasion and promotes metastatic spread through a pathway independent of IAP inhibition of cell death[26].

In view of apoptotic events, these results indicate that the transition from well-differentiated (PTC) to undifferentiated carcinoma(ATC) is accompanied by down-regulation of antiapoptotic molecules, galectin-3 and Bcl-2, and up-regulation of the apoptosis inhibitor, survivin, which may at least in part, further facilitate the ability of malignant thyroid cell to escape apoptotic cell death despite high expression. Indeed, molecular studies have demonstrated that down-regulation of survivin in thyroid cancer cell lines increased apoptotic cell death and sensitivity to chemotherapeutic agents[36]. A higher incidence of survivin expression in anaplastic than in papillary carcinomas was first reported by Ito *et al*. suggesting that this protein is related to dedifferentiation of thyroid carcinoma and was thereafter confirmed[37].

The antiapoptotic functions of cytoplasmic galectin-3 has been consistently shown in many types of cancer cells, including breast, prostate, thyroid, bladder, colorectal, pancreatic, gastric, myeloid leukemia, neuroblastoma, and some B-cell lymphoma[38].Numerous studies have indicated that galectin-3 is involved in multiple stages of cancer progression and metastasis and mayrender anticancer activities in several ways. Califice *et al*l observation that Gal-3 was consistently excluded from the nucleus in prostate cancer cells but not in nontumoral prostatic glands, indicates that Gal-3 can play antitumor activities when present in the nucleus, whereas it can favor tumor progression when expressed in the cytoplasm[25].

In conclusion, the intracellular (cytoplasmic) galectin-3 is antiapoptotic providing survival advantage to cancer cells, galectin-3 promotes tumor neoangiogenesis and tumor–endothelial cell interactions required for metastasis are believed to be mediated by endothelium-associated galectin-3. We thought that tumor cell secreted galectin-3 induces apoptosis of cancer-infiltrating T-cells possibly promoting immune escape during tumor progression. The molecular mechanisms underlying this observation remain to be investigated, and could constitute the basis of future anticancer therapeutic strategies

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