MINI-REVIEW

Research Progress on the Livin Gene and Osteosarcomas

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Abstract

Osteosarcoma is a common malignant tumor of bone, but mechanisms underlying its development are still unclear. At present, it is believed that the inhibition of normal apoptotic mechanisms is one of the reasons for the development of tumors, so specific stimulation of tumor cell apoptosis can be considered as an important therapeutic method. Livin, as a member of the newly discovered inhibitor of apoptosis proteins (IAPs) family, has specifically high expression in tumor tissues and can inhibit tumor cell apoptosis through multiple ways, which can become a new target for malignant tumor treatment (including osteosarcoma) and might of great significance in the clinical diagnosis of tumors and the screening of anti-tumor agents and carcinoma treatment.

Keywords: Livin gene - osteosarcoma - cellular apoptosis - target therapy

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Introduction

Osteosarcoma, as a most common primary malignant tumor of bones, occurs frequently in adolescents with high malignant severity (Bao et al., 2013; He et al., 2013; Jia et al., 2013; Jiang et al., 2013; Jin et al., 2013). It is commonly found on distal femur and proximal tibia with high rates of recurrence and metastasis and poor prognosis. The developmental mechanism is still unclear, however, with the progression of molecular genetics and biology of tumors, the inhibition of conventional apoptotic system had been regarded as the primary cause of tumor development. Cellular apoptosis system exerts great function in the development and progression of osteosarcoma, but no definite molecular target has been found recently. The critical role inhibitor of apoptosis proteins (IAPs) play in regulating cellular apoptosis has become the focus of present anti-tumor research. So far, 8 members of IAPs have been discovered in human body including NAIP, XIAP (ILP-1/MIHA), cIAP-1 (HIAP-2/MIHB), Livin (ML-IAP/KIAP), Survivin (TIAP), cIAP-2 (HIAP-1/MI-HC), ILP-2 (Ts-IAP) and Apollon (Bruce) (Zou et al., 2014), in which Livin can be applied as a new target for malignant tumor treatment (including osteosarcoma) due to its specifically high expression in tumor tissues.

Structure and Function of Livin

Livin gene is located in the q13.3 of the 20th chromosome. It is 4.6kb in length and has 7 exons. Livin protein amino terminal consists of 1~3 baculovirus IAP baculovirus IAP repeat domain (BIR) in length similar to 70 amino acid in conventional length, while carboxyl terminal of 1 ring domain (RING). BIR includes 4 α helix and 1 triplex anti-parallel β lamella, whose framework is of critical importance on anti-apoptosis activity while RING finger domain mediates the distribution of Livin genes on sub-cellular level (Chen et al., 2013).

Anti-cellular Apoptosis Function of Livin

Livin has obvious inhibiting function on cell apoptosis mediated by death receptor, mitochondria and chemotherapeutic agents. This anti-cell apoptosis effect is primarily realized by inhibiting Caspase activity and activating TAK1/JNK1 signal pathways (Li et al., 2013).

Livin-inhibiting-Caspase pathway

Livin can combine with downstream effect Caspase that can mediate cell apoptosis, which means to combine with Caspase-3 and Caspase-7 in activated form and inhibit the activities. Livin can inhibit APaf-1, cytochrome C and dATP-induced Caspase-9 activating effect when combined with Caspase-9 in unprocessed or fractured form. Livin can also directly disturb the Procaspase-9 or caspase-9 activity on the starting point of apoptosis.

TAK1/JNK1 signal transduction activating pathway

Livin can activate MAP kinase JNK1 and JNK2, but has no function on JNK3. Livin has stronger activating function on JNK1 than on JNK2, which is also a major pathway for Livin against tumor necrosis factor-α (TNF-α) and ICE-mediated cell apoptosis. JNK proteins can be directly activated by MKK4/MKK7, but the activation of Livin on JNK1 is dependent on TAB1/TAK1

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pathway instead of MKK4/MKK7 signal transduction. TAK1, as a MAP3 kinase on upstream, can activate JNK1 under the stimulation of TGFβl. TAB1, as the co-activator of TAK1, has no activating function on JNK1, but can improve TAK1-mediated JNK1 activation. Livin can combine with TAB1 and further activate TAK1.

Second mitochondrial activator of caspases (SMAC)-mediated negative regulation

The mechanism of SMAC-mediated negative regulation on Livin anti-apoptosis reaches its aim by specifically combining the SMAC and bioactive peptide fragments and the BIR structural domain so as to inhibit the combination of Livin and Caspase.

Tissue Distribution and Expression of Livin in Osteosarcoma

Disorder of cellular growth and apoptosis pathways is beneficial to the growth and invasion of tumors, and the abnormal expression of Livin in osteosarcoma can directly improve the escape of tumor cells from growth surveillance and then inhibit tumor cell apoptosis. Abd-Elrahman et al. (2013) pointed out that cellular apoptosis system might play an important role in the development and progression of osteosarcoma, but no effective functional target was found. Inhibition of tumor cell apoptosis system is one of the primary motivating factors for the development and progression of tumors, which is also in close association with tumor properties such as the drug-tolerance of tumor cells and radio-chemotherapeutic survivability, etc..

Guo et al. (2013) discovered that Livin was mainly located in cytoplasm of tumor cells in filiform, and had different degrees of expression in few cell nucleus and cytoplasm, but had no expression in tumor stroma and pericarcinomatous tissue. Liu et al. (2013) applied immunohistochemistry to detect the expression of apoptosis-inhibiting protein Livin in 45 patients with osteosarcoma and 30 with osteochondroma, whose results demonstrated that Livin rarely existed in tissues of osteochondroma but with over-expression in osteosracoma, indicating that Livin expression had specificity in osteosarcoma. Further analysis on the clinical biological indexes of osteosarcoma showed that Livin expression was evidently higher in invasive metastatic tissues than in non-invasive metastatic ones, suggesting that the higher the Livin expression in osteosracoma, the stronger the inhibiting effect and the longer the survival rate of tumor cells, which was predicated to be in certain association with the lung metastasis of osteosarcoma.

Positive Livin expression is characterized by low differentiation, strong invasiveness and tendency of migration. Study showed that Caspase-3 was in evidently negative correlation with Livin expression because Caspase-3 was located in the center of apoptotic regulation system, whose low expression could trigger irregular apoptosis of osteosacoma cells and enable the rapid tumor proliferation without obstacles, becoming the major reason that osteosarcoma was higher in malignant severity than routine tumors from epithelial tissues (Nedelcu et al., 2008). Consequently, patients who had limb salvage surgeries will confront with the risks of postoperative recurrence and metastasis of tumors.

The research of Xie et al. (2011) verify that Livin had consistency with the microvessel density (MVD) of osteosarcoma, indicating that Livin expression and functional regulation could regulate physiological reparative or pathological angiogenesis, which had potential effect on the infiltration and metastasis of osteosarcoma. This conclusion was further proved by Li et al. (2012) by experiments, in which it was demonstrated that Livin expression had no significant association with the gender, age and tumor types of patients, but had certain relationship with the Enneking surgical stagings, and the positive expression rates of Livin were 30.5% and 67.6% in patients in stage I~IIA and IIB~III, respectively, and the difference was significant. It is well known that the later the Enneking staging is, the poorer the prognosis and the shorter the survival time, therefore, it is also believed that the positive Livin expression rate is in potential connection with patients' prognosis and the detection of Livin expression may play a critical role on the prognostic evaluation of osteosarcoma. In consequence, more comprehensive surgical methods should be conducted to patients with positive Livin expression, in which radiochemotherapy should be performed after surgeries to reduce the follow-up intervals and closely observe disease progression so as to greatly decrease the recurrent rate and prolong the survival time of patients with osteosarcoma.

Livin and Osteosarcoma Treatment

At present, immunotherapy and gene therapy have been more superior because of the severe traumas caused by conventional surgeries and the lower recovery rate of radiotherapies and chemotherapies with more toxicities and adverse responses as well as the potential drug-tolerance in the treatment of tumors, especially in osteosarcoma. A study revealed that osteosarcoma U20S cells with high expression of Livin had increased reproductive growth rate and had rapid speed and short time of cell proliferation after being transfected, indicating that Livin could promote osteosarcoma cell proliferation.

Livin can become a new target for osteosarcoma treatment because the weakened expression of Livin be some agents could inhibit tumor cell proliferatuion. Presently, research on osteosracoma with Livin as target basically focuses on silent Livin gene and inhibiting Livin expression and function. Li et al. (2007) adopted RNA interfering technique in vitro to disturb osteosarcoma U2OS cells and found that cell growth and invasive function had been evidently inhibited and cell growth had been stopped at phase G0/G1, which could improve the chemotherapeutic sensibility of osteosarcoma to cis-platinum and reduce the factors (Cyclin D1, Bcl-2, MMP-2 and MMP-9) closely associated with osteosarcoma growth and metastasis. Wang et al. (2011) utilized differentiated-dosage of low molecular weight heparin (LMWH) in vitro on osteosarcoma MG-63 cells and discovered that LMWH could inhibit the growth and proliferation of osteosarcoma cells in specific time and dosage-dependence. With the

increase of heparin dosage and the prolong of action time, the apoptotic ratio of osteosarcoma MG-63 cells would increased markedly, indicating that LMWH had anti-tumor activity in vitro, and its action was predicated to be achieved by inhibiting Livin to influence the cell mitosis so as to improve the tumor cell apoptosis. A study verified that nuclear transcription factor (NF-kB) inhibitor could down-regulate Livin expression and then up-regulate Caspase expression by inhibiting NF-kB activity to improve osteosarcoma apoptosis so as to provide more new pathways for osteosarcoma treatment (Ma et al., 2011). In addition, the comprehensive therapeutic methods consisting of new-type gene target therapy and immunotherapy advocated by other scholars are still in in vitro experimental phase at present, which need more clinical experiments to be further proved to provide more effective new pathways for tumor treatment.

Conclusions and Prospects

Tumors are characterized by rapid proliferation and low apoptotic rate, therefore, specific stimulation on tumor cell apoptosis is an important way for tumor therapy. At present, great achievements have been made on Livin, however, the relationship between Livin and tumors still has numerous levels to be further explored, such as the explicit mechanism of Livin in inhibiting tumor apoptosis, relationship of Livin and oncogenes, anti-oncogenes and other apoptosis-related genes, association of Livin with cell signal transduction as well as multiple therapeutic measures specific for Livin genes, etc.. Additionally, problems like whether gene therapy with Livin as the target gene and whether Livin can be used as a new tumor marker in clinic as well as the connection and mechanism of Livin and chemotherapeutic-induced drug tolerance are still unclear and needed to be further studied.

Study on the structure and function is of great significance for the clinical diagnosis and the screening of anti-tumor agents and therapeutic methods due to the specific high-expression of Livin in some tumors. It is firmly believed that with the deepened research of Livin, its expression and anti-tumor mechanism in tumor tissues can be further illustrated, which is beneficial to the development and gradual application of Livin-associated antibodies and vaccines so as to provide new pathway for the diagnosis and treatment of tumors, especially osteosarcoma.

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