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Mini-review

Several new targets of antitumor agents

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KEY WORDS neoplasms; antineoplastic agents; alpha fetoproteins; oncogenes; cell differentiation; apoptosis; phytogenic antineoplastic agents

ABSTRACT Alpha-fetoprotein (AFP), as a hepatoma-promoting factor, has become a new target of anti-hepatoma agents. It is a new approach for the treatment of tumors to inhibit or block oncogene expression. Informational drugs are being developed for gene therapy applications as inhibitors of oncogene expression. The induction of tumor cell differentiation is another new strategy of drug therapy of tumors. Common action mode of many antitumor drugs is to induce apoptosis of tumor cells. Suicide genes, as targeting therapy of tumors, improve the present chemotherapy, exhibiting broad application prospects.

In many conventional chemotherapeutic regimens of tumors, drugs display their activities on the DNA level. But, these drugs are often deleterious for other rapidly dividing cells and cause the well-known toxicities. Therefore attempts aimed at other pharmacological targets are especially interesting. In recent years, an important development in the field of cancer pharmacology is the finding of some new targets of antitumor agents[1,2]. Based on our investigations, several new targets will be introduced in the present review.

Alpha-fetoprotein (AFP)

Standard therapy of primary hepatocellular carcinoma (PHC) was surgical resection. However, high recurrence rates have been disappointing. A major problem of chemotherapy and radiotherapy for PHC is still their toxicities. There were no data from controlled studies available which would allow an assessment of efficacy of immunotherapy for PHC[3]. It is pressing to find new methods to improve therapeutic efficacy and reduce extrahepatic side effects. It has been recognized that AFP, as an oncofetal antigen, re-expresses in large amounts in adult hepatoma cells and serves clinically useful purposes as a PHC marker assay. However, its biological activities are still far from clear. *In vivo* application of AFP in rodents increases their susceptibility to various experimental tumors demonstrated by greater tumor sizes, longer periods for tumor regression, and an increased number of tumor progressions. But, the heightened susceptibility can be explained by immunosuppression of AFP. Our studies indicated that AFP inhibited the proliferation of splenic lymphocytes[4], one-way mixed lymphocyte reaction, and interleukin-6 production of spleen cells (to be published). Suppressor T (T_s)-cell activity was also enhanced by treatment of AFP (to be published). On the other hand, our laboratory found that AFP also directly functioned as a PHC-promoting agent. Human AFP stimulated the growth of mouse ascites hepatoma-22 (H-22) cells. When H-22 cells were incubated with human serum albumin (HSA), no obvious influence on H-22 cell growth was obtained, indicating that growth-stimulatory effect of AFP was not a nonspecific nutrition of this protein on cultured cells. To further confirm the specificity of action of AFP, H-22 cells were incubated with combined AFP and anti-AFP antibody. The growth-stimulatory effect of AFP was obviously abolished by anti-AFP antibody[5]. Taking all our investigations, it is strongly inferred that AFP contributes to the generation and development of PHC and is an important target of anti-PHC agents[6]. Suppression of the gene expression and biological activities of AFP by some bioactive substances will become a new strategy of the treatment of PHC[7].

L-4-Oxalysine (Oxa) is a new antitumor and immunoregulatory agent of natural origin found first in our Institute[8-10]. Our laboratory found that Oxa 12.5 $mg \cdot L^{-1}$ had no marked antagonistic action on the growth stimulation of H-22 cells by AFP. But, when Oxa was used in combination with 5-fluorouracil 3.1 $mg \cdot L^{-1}$, the percent

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of suppression on H-22 cell growth stimulated by AFP was increased[11,12]. On the other hand, Oxa produced an inhibitory influence on the activity of T_s cells induced by AFP[11,12]. AFP-suppressed splenocyte proliferation, one-way mixed lymphocyte reaction, and interleukin-6 production were also antagonized by the treatment of Oxa (to be published). Using *in situ* hybridization technique with digoxigenin-labeled AFP cDNA probes and immunocytochemistry method, we found that essentially all human hepatoma BEL-7404 cells contained AFP mRNA and AFP protein in the cytoplasm. Exposure of hepatoma cells to Oxa resulted in an obvious decrease of AFP mRNA and AFP protein, indicating that Oxa inhibited AFP gene expression in human hepatoma cells[13]. Therefore, according to the data from our laboratory, it is suggested that anti-hepatoma activity of Oxa is associated with its influence on AFP and Oxa has the potential of being developed as a new anti-PHC drug based on AFP target. In addition, previous investigation indicated that retinoic acid (RA), a differentiation inducer, suppressed AFP gene expression in human hepatoma SMMC-7721 cells. Synergical inhibition on AFP gene expression was also obtained when RA was combined with selenium. Moreover, anti-AFP activity of RA was proportional to its therapeutic effect on liver cancer[14].

Oncogenes

The discovery of oncogenes has led to the development of tumor therapies based on affecting the activated oncogenes by some bioactive compounds.

Lycobetaine (Lyc), a new antitumor agent of natural origin, has been shown to decrease the expression of c-*myc*, N-*ras*, and β_2-microglobulin genes in human leukemia cell line HL-60 cells[15]. After the treatment with Lyc, the sensitivity of c-*myc*, N-*ras*, and β_2-microglobulin genes to DNase I was lowered, while that of c-*myb* oncogenes did not change obviously, indicating that influence of Lyc on oncogenes was related to different active structural states of oncogenes[16].

Homoharringtonine (Hom) was found to have therapeutic effect for acute leukemia as well as some solid tumors in combination use. In molecular hybridization experiments, Hom reduced the content of c-*myc* RNA in the cytoplasm and accelerated the degradation of c-*myc* mRNA in the cytoplasm. But the agent exhibited no marked influence on the expression of c-*myc* oncogene at transcriptional level[16,17].

Strategies aimed at affecting oncogene expression also include application of informational drugs (ID) such as antisense oligodeoxynucleotides (AODN), antisense oligonucleotides (AON), and ribozymes (RZ). They can block oncogene expression at different levels. AODN and AON are designed to bind to double-stranded DNA, resulting in triple helix formation and reduction of mRNA synthesis or are designed to specifically bind to RNA, resulting in a translational arrest. RZ is a naturally occuring RNA enzyme that catalyzes RNA cleavage and RNA splicing reactions in a sequence-specific manner. RZ that catalyzes RNA cleavage is being developed for gene therapy application as inhibitor of the expression of oncogenes[18,19]. A major problem is the stability of the therapeutic ID *in vivo* as well as the specific cellular targeting.

Differentiation induction of tumor cells

Since clinical application of RA for the treatment of acute promyelocytic leukemia, studies on differentiation inducers have always attracted the attention of the world's scientists[20].

Anordrin (Ano)is a postcoital contraceptive developed in China and possesses antiestrogenic properties[21]. Our laboratory found that α-Ano exhibited potent antitumor activities[22,23]. Human leukemia HL-60 cell line is a most frequently employed *in vitro* model to evaluate the ability of various differentiating agents. It was shown by NBT dye reduction method and morphological observation that α-Ano 10 $\mu mol \cdot L^{-1}$ induced HL-60 cell differentiation into more mature cells. Meanwhile, the membrane fluidity of HL-60 cells and Ca^{2+} content in the cytoplasm were decreased. The activity of alpha-phosphorylase was enhanced. The expression of c-*myc* and N-*ras* oncogenes in HL-60 cells was also inhibited[24].

In addition to RA and α-Ano, two new synthetic analogs of RA, 4-(ethoxycarbophenyl) retinamide and 4-(hydroxycarbophenyl) retinamide could also induce HL-60 cells to differentiate to more mature cells with functional characteristics of granulocytes[25]. Icariin (Ica) increased the reduction of NBT and reduced nuclear area in HL-60 cells. The nuclear morphology of Ica-treated HL-60 cells was

changed into rod or lobulated shape and the volume of nuclei reduced. These results suggest that Ica may also induce differentiation of HL-60 cells[26].

Apoptotic process of tumor cells

Cell death can occur by two quite distinct mechanisms, necrosis and apoptosis. Apoptosis is a mode of cell death that occurs under normal physiologic conditions. Opportunities are now opening up where this basic knowledge can be translated into new approaches to the development of antitumor drugs (Tab 1)[27].

Tab 1. Differential features and significance of necrosis and apoptosis.

Necrosis	Apoptosis
Morphological features	
Loss of membrane integrity	Membrane blebbing, but no loss of integrity
Flocculation of chromatin	Aggregation of chromatin at the nuclear membrane
Swelling and lysis of the cell	Cell shrinkage
No vesicle formation, complete lysis	Formation of membrane bound vesicles (apoptotic bodies)
Swelling of organelles	No swelling of organelles, organelles remain intact
Biochemical features	
Loss of regulation of ion homeostasis	Tightly regulated process involving activation and enzymatic steps
No energy requirement (passive)	Energy (ATP)-dependent (active)
Random digestion of DNA (smear of DNA after agarose gel electrophoresis)	Non-random mono- and oligonucleosomal length fragmentation of DNA (ladder pattern after agarose gel electrophoresis)
Postlytic DNA fragmentation	Prelytic DNA fragmentation
Physiological significance	
Death of cell groups	Death of single, individual cells
Evoked by non-physiological disturbances	Induced by physiological stimuli
Phagocytosis by macrophages	Phagocytosis by adjacent cells or macrophages
Significant inflammatory response	No inflammatory response

α-Ano 50 $\mu mol \cdot L^{-1}$ resulted in marked morphological changes of leukemia K562 or HL-60 cells including condensed chromatin, nuclear fragmentation, and reduction in volume. Agarose gel electrophoresis of DNA from cells treated with α-Ano revealed "ladder" pattern, a typical feature of apoptosis. At concentration of 50 $\mu mol \cdot L^{-1}$, α-Ano stimulated 52 % apoptosis of K562 cells and 61 % of HL-60 cells as determined by flow cytometry. The S-phase cells were more susceptible to apoptosis. Despite extensive cleavage of DNA and nuclear fragmentation, the cell membrane of α-Ano-treated cells remained intact, excluding trypan blue[28,29]. Apoptosis is often abrogated or delayed by inhibitors of protein synthesis, such as cycloheximide (Cic). However, apoptosis of K562 cells induced by α-Ano was not prevented by Cic, ie, α-Ano-induced apoptosis of K562 cells was independent of new protein synthesis[30].

The elimination of tumor cells by intracellular expression of suicide molecules is also an effective method to control malignant tumors. When suicide genes such as herpes simplex virus thymidine kinase (HSV-tk) gene, *E coli* cytosine deaminase (EC-cd) gene, and varicella-zoster virus thymidine kinase (VZV-tk) gene are transduced into tumor cells, suicide molecules expressed by these genes enzymatically covert prodrugs such as ganciclovir, 5-fluorocytosine, and 6-methoxypurine arabinoside to potent inhibitors of DNA synthesis, resulting in the selective apoptosis of transducted tumor cells. This innovative approach has been explored for the treatment of tumors[31,32].

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抗肿瘤药的几个新靶点

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关键词 肿瘤；抗肿瘤药；甲胎蛋白；癌基因；细胞分化；细胞凋亡；植物源的抗肿瘤药

摘要 甲胎蛋白作为肝癌生长的促进因子，是抗肝癌药的新靶点. 用药物抑制或封闭癌基因表达为治疗肿瘤开辟了新途径. 诱导肿瘤细胞向正常细胞分化已成为肿瘤药物治疗的又一崭新策略. 诱导肿瘤细胞凋亡是许多抗肿瘤药的共同作用方式；用自杀基因靶向治疗肿瘤优化了目前的化学治疗，具有广阔的应用前景.