

The Use of AFP-Complexes to Induce Apoptosis in Cancer Cells

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Abstract: An anticancer “magic bullet” should have both efficacy and specificity parts. We have used an effective Apoptosis Inducer to trigger the apoptosis. Alpha-fetoprotein was used to deliver Apoptosis Inducers specifically to cancer cells. The AFP-AI complex inhibited tumor growth in mice, enlarged mice life survival and has shown a 50% response in patients with metastatic colorectal cancer.

Keywords: Cancer, apoptosis inducer, targeted delivery, alpha-fetoprotein, multi drug resistance.

INTRODUCTION

Cancer is the proliferation/apoptosis overbalance. Proliferation and apoptosis are different systems and are used completely differently like the car gas and brake systems (Fig. 1). Traditional anticancer drugs mainly aim at suppressing proliferation by any mean (more than 700 molecular targeted agents are currently in trials), but directly activating apoptosis is a better idea (less than 30 agents in trials) [1, 2]. Apoptosis is responsible for recycling billions of cells everyday in the body. Cancer cells accumulate mutations in both proliferation and apoptosis systems, but crucial mutations in the apoptosis system help them to avoid the natural suicidal pathway. To overcome the broken apoptosis elements, the drug should act “downstream” of mutated apoptosis cascade elements (Fig. 1). To trigger the apoptosis, a drug needs to: 1. be specific to cancer cell, 2. provide direct Apoptosis Inducer (AI) internalization, 3. Use AI acting “downstream” of the broken apoptosis cascade element. For those purposes, several combinations can be obtained with nanoparticles, immunoliposomes, cell-penetrating peptides, small molecule AIs, etc. We have prepared a non-covalent complex of alpha-fetoprotein (AFP) that can specifically deliver AI to cancer cells and act on functional executive apoptosis elements.

EFFECTIVE KILLING: DON'T REINVENT THE WHEEL – FIX THE BRAKES

Many cancer drugs are not optimal and often ineffective since they aim at the targets within the proliferation system (for example, DNA) which might not lead to the cancer cell death due to a frequent mutation of the p53 protein or other mutations in apoptosis pathways (Fig. 1) [3].

The problem of specific and effective elimination of definite cells in the body is already solved in Nature. Cytotoxic T lymphocytes (CTL), for example, use this mechanism. They first recognize wrong cell by membrane antigens and then use perforin for the internalization of granzyme B that activates caspases – the final enzymes within the apoptosis cascade (Fig. 1) [4]. Granzyme B is a direct apoptosis inducer and is not dependent on “upstream” cancer cell mutations such as p53 and others. For this reason, CTL can

“fix the brakes” meaning they are using undamaged apoptosis elements inside cancer cells (Fig. 1). Traditional chemotherapy targeting proliferation system elements (for example, doxorubicin: DNA) can be attributed to indirect apoptosis inducers because it can lead to the following next activation of apoptosis. Non repairable DNA mutations caused by doxorubicin lead to p53-dependant apoptosis in normal cells. Direct apoptosis inducers on the opposite, trigger apoptosis themselves. The closer direct apoptosis inducer can act on a late apoptosis event less chances are that cancer cell mutations might prevent its action. The ideal situation is that caspase activators such as the PAC-1 molecule, being the last target in the cascade, will activate procaspase 3 thus triggering caspase 3 actions and demonstrate an effective anticancer action [5].

We tried to copy the effective natural CTL “know-how” to find and kill cancer cells by combining our lessons learned from CTL and using the known biological tools.

A membrane is considered being the edge between life and death. Damage in the membrane of mitochondria, endoplasmic reticulum, lysosome or peroxisome can lead to the whole eukaryotic cell death. The membrane of mitochondria was chosen as a target because mitochondrion is a “point of no return” in the intrinsic apoptosis pathway [6]. We have used proven apoptosis inducers acting on mitochondrion such as atractyloside, betulinic acid [7, 8] and an endoplasmic reticulum apoptosis inducer thapsigargin [9]. Within the intrinsic apoptosis pathway, the mitochondrion acts downstream of the p53 protein. P53 is inactive in more than 50% of cancer cells and often leads to multi drug resistance (MDR) [10]. Atractyloside was shown to induce apoptosis in p53-unfunctional HuH-7 cancer cells [11].

Since many of the agents do not cross the plasma membrane, we had to use agents combined to a special delivery system in order to induce their internalization of those agents with a special delivery system. Moreover, this delivery system should have the ability to select cancer cells and avoid healthy tissues.

SPECIFIC DELIVERY

AFP may be responsible for embryotoxic and teratogenic action of some drugs, pollutants and chemicals. One of the

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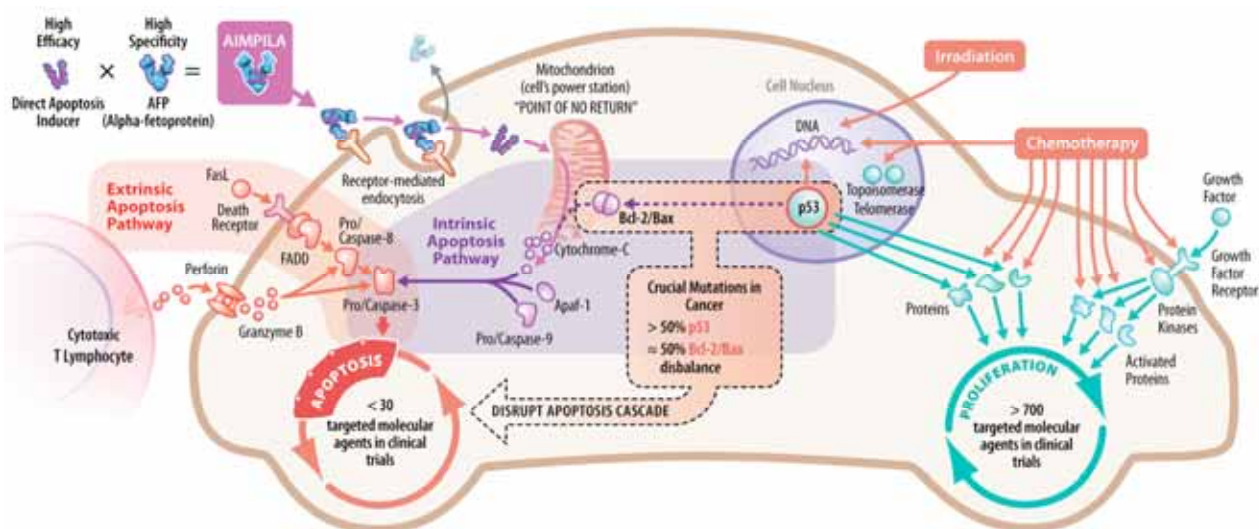


Fig. (1). Proliferation and apoptosis systems and associated anticancer drug targets. Illustration (c) 2008 AXS Biomedical Animation Studio. Used with permission.

explanations of this phenomenon is that AFP has the ability to take toxins from mother's blood and bring it to the embryo [12]. In such case, AFP should compete with the mother's albumin for binding to the toxin. Among many other substrates AFP has the strongest binding affinity to polyunsaturated fatty acids (PUFA). After the AFP-PUFA complex endocytosis by embryo cells the PUFA is internalized [13]. We have substituted the PUFA in the porcine AFP-PUFA complex for atractyloside and have obtained the natural effective delivery system for the powerful direct AI.

A lot of AFP conjugates were used for the targeted delivery of different toxins to cancer cells, for example, AFP-esperramicin A1 [14].

We use the non-covalent complex that is closer to natural protein form and will not provoke immune attack as AFP conjugates can possibly do. In this case we can rely on the pharmacokinetic data obtained for natural human AFP injectable drug named "Alfetin" that was shown to have 3-5 days of half-life time in the human body [15]. Pre-binding of AFP (MW=70 kDa) to atractyloside (MW=0.8 kDa) in 1:1-2 molar ratio possibly do not change pure AFP pharmacokinetic profile.

DISCUSSION

AFP is a known oncology marker [16, 17, 18]. For the addressed delivery of the AI, we have used AFP which has highly specific receptors re-expressed on the majority of cancer cells [19] and is up taken by them [20].

AFP-mediated delivery of dioxin to cancer cells having AFP receptor (AFPR) was 200-1400 times more effective than dioxin alone [12]. The environmental toxin dioxin is itself carcinogenic [21] and is not a direct AI, so its anticancer application is problematic.

An injectable complex of AFP with an antifungal drug amphotericin B was used to treat cancer [22, 23]. Amphotericin B does not act on mitochondrion [24] and hence is not as effective as direct AI.

We non-covalently bound AFP to a proven direct AI - atractyloside which is toxic to the mitochondria (Fig. 1). The AFP-AI complex (Aimpila™) was prepared as described in [25]. Briefly, the porcine embryo blood and amniotic fluid were collected, purified and concentrated with 50 kDa MWCO ultrafiltration module, AFP was extracted by butanol, diafiltrated, complexed with atractyloside in 1:1-2 molar ratios and lyophilized.

The Aimpila™ (0.02 mg in 0.2 ml of oil/day) being given orally has shown to inhibit tumor growth in the mice p388 leucosis model by 85% within 25 days and enlarged mice life survival by 36% [25].

The p388 leucosis model for Aimpila™ in mice was taken because was used before for AFP-drug conjugates testing [14].

AFP is known to be resistant to proteolytic attack from enzymes such as trypsin [26]. The oral route for peptide and protein drug delivery for several drugs was shown before [27]. The protein of the similar size as AFP - recombinant interferon - is absorbed from rectal suppositories and was effective to suppress replication of hepatotropic viruses in the liver of children with hepatitis B and C [28]. The oil added to the drug formulation probably helped preventing Aimpila™ from degradation in GI tract and improved its absorption in the intestine.

An encapsulated form of Aimpila™ (0.6 mg/day) was used as monotherapy in patients with metastatic colorectal cancer (mCRC). mCRC was chosen because colon cancers have AFPR [19], and it was known to have 76,8% mutations of the p53 [3].

0.012 mg/day of the atractyloside, or 1/millionth of the pig's oral LD50=25-100 mg/kg were supposed to be non-toxic within Aimpila™ [26].

CT-scans before and after the 8 weeks of treatment has shown that 6 out of 12 mCRC patients (50%) have responded to a suboptimal dose of Aimpila™ [29]. Two (2) of these 6 patients have shown full reduction of metastasis, one (1) patient a 73% reduction of the size of his metastasis and

three (3) patients were stabilized. No significant side effects, quick growth inhibition and tumor reduction were reported in that human study. This could be attributed to the way metastases were treated by using a direct apoptosis mode of Aimpila™ action.

Breast, liver, lung, ovarian, stomach, prostate and other cancers are AFPR-positive [19] and could potentially be treated with Aimpila™.

The data provided here support the idea of the necessity of the right combination of specific and effective parts to form an anticancer “magic bullet”. This means a feature which inevitably induces apoptosis in cancer cells and avoids potential mutation pathways. In addition, a second feature should be added to deliver the drug only to cancer cells and internalize it for optimal results.

CONCLUSION

Aimpila™ meets the necessary and sufficient conditions to be considered as an anticancer “magic bullet” because of its high specificity to AFPR-positive cancer cells and because it internalizes atractyloside thanks to the AFPR-mediated endocytosis. Moreover, it has high efficacy in killing cancer cells due to direct apoptosis inducer action.

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