

Translational Medicine 2015: Survivin siRNA nano particles are capable of inhibiting cancer cell growth both in vitro and in vivo - Suoqin Tang - PLA General Hospital

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Abstract

Since the stability of siRNA molecules inside the blood and efficiency of siRNA distribution into target organs or tissues subsequent systemic management are the main issues that limit requests of siRNA in human patients, we attempt to explore if siRNA liposome entrapment works within the development of novel therapeutics. Our study aims to gauge the therapeutic effect of survivin siRNA nano particles, on cancer of the liver, carcinoma and cervical cancer both in vitro and in vivo. First, sequences of survive in siRNA we designed had been screened for his or her efficacy, and therefore the best one was chosen for subsequent study. Second, we've tested the biological effect of survivin siRNA nanoparticles on neoplastic cell lines in vitro, and resulted that, the survivin mRNA and its protein expression was significantly inhibited in MHCC-97H cells, HeLa cells and LoVo cells, also proliferation of these cell lines was inhibited and apoptosis was promoted. Third, subcutaneous xenograft Balb/c nude mice of MHCC-97H cells, HeLa cells or LoVo cells were established. Survivin siRNA nanoparticles (70.7±29.077 nm in size), with dosage of 3 mg/kg survivin siRNA was given via local or injection, twice every week continuously for four weeks. As results, major tumor growth reserve both in local injection and venous injection was experiential, compared to regulate mice who received scribbled siRNA nan particles at an equivalent dosage, with inhibition rate of 32.22% and 36.67%, at the top of study in venous injection and native injection respectively, the relative tumor volume in mice with local injection showed significant but control group from 10 days after first injection ($P<0.05$), till 31 days, it had been also an equivalent situation in mice with venous injection compared to regulate, ($P<0.05$) except day 17, survivin mRNA and its protein were down regulated at the top of study, compared to regulate group. Our study also showed some inhibition activity of survivin siRNA nanoparticles in subcutaneous xenograft Balb/c nude mice of HeLa or LoVo cells, survivin mRNA and protein expression was all down regulated in tumor tissue compared to regulate. We considered the survivin siRNA with Cy3 florescence and injection to nude mice with MHCC-97H cells, and located

intense Cy3 delivery in tumor mass, spleen and liver, but scant delivery in heart, bone marrow, brain, lung, and alimentary canal. Our study revealed that survivin siRNA nanoparticles were capable of inhibiting tumor growth both in vitro and in vivo.

Keywords: cancer therapy, gene delivery, liposome, nanoparticles.

Introduction: RNAi technology has recently been developed as a possible therapeutic agent. Compared with antisense oligonucleotides, siRNAs are more immune to nuclease degradation and show a more prolonged therapeutic effect. RNAi works in both cell lines and various primary cells, if it's properly delivered. It's broadly, yet specifically, applicable to any target gene with which the sequence is understood. The promise that siRNA can specifically down regulate 'undruggable' gene products brings hope to the 'incurable' diseases and ushers during a new era of pharmaceutical science. This review summarizes different signaling pathways inhibited by siRNA and therefore the advantages within the usage of targeted siRNA delivery systems.

A. siRNA for Cancer Treatment: Angiogenesis may be a main factor that regulates tumor growth, invasion and metastasis. The vascular endothelial protein (VEGF) family has been reported as a key mediator of those processes. VEGF, a glycoprotein, can activate the signaling pathway to reinforce endothelial cell growth, differentiation and migration and protect the pre-existing vasculature from death. Activation of VEGFR-1 or VEGFR-2 can cause cell survival, proliferation or migration via several different pathways. First, Ras pathway is activated to stimulate cell proliferation and survival via the mitogen-activated protein-kinase (MAPK) cascade. Secondly, VEGF also can phosphorylate phospholipase C (PLC- γ), which activates protein kinase C and triggers the MAPK pathway. Thirdly, the AKT pathway is phosphorylated through VEGFR activation during a PI3K-dependent manner. Finally, focal adhesion kinase (FAK) activation is mediated via the C-

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terminal tail of VEGFR-2 and is required for cell migration.

siRNA's targeting oncogenes that are involved in survival or antiapoptosis show great therapeutic potential for cancer therapy. The Wnt pathway plays a crucial role in carcinogenesis. Wnt signaling is transduced through Wnt receptors to the beta-catenin-Tcf pathway, the c-Jun-N-terminal kinase (JNK) pathway or the Ca (2+)-releasing pathway. Wnt/beta-catenin signaling pathway is much activated and leads to beta-catenin accumulation during a sort of human cancers. Anti-Wnt-1 siRNA induced apoptosis in MCF-7 carcinoma cell line. siRNAs blocking Wnt pathway have also been used to target cancerous stem cells in non-small cell carcinoma (NSCLC), which has limited response to single-agent chemotherapy. These results suggest that siRNA inhibiting Wnt pathway may function as a possible anticancer agent.

Drug resistance of tumor cells is one among the main limitations of chemotherapy. Subpopulations of tumor cells immune to a given chemotherapeutic drug leave continued tumor growth. Multiple drug resistance (MDR1) gene is directly associated with the drug resistance to vinca alkaloids (vinblastine, vincristine), anthracyclins (adriamycin, daunorubicin), etoposide and paclitaxel. For enhancing the therapeutic effect of those chemotherapeutic agents, siRNAs were designed to inhibit MDR1 expression and convert the tumor back to the drug-sensitive state.

Effective strategies to deliver siRNA systematically into solid or malignant tumor should fulfill a minimum of five requirements: protection of siRNA from degradation and rapid clearance; prolonged circulation time after administration; efficient uptake of siRNA by the tumor; cellular uptake of siRNA; and endosomal release of siRNA into the cytoplasm. Some viral vectors can effectively deliver their genomes into the tumor cells and express short hairpin RNA (shRNA) for gene silencing. But none of the viral vectors can efficiently accumulate within the tumor after systemic administration. Their strong immunogenicity and other questions of safety also are concerns.

Therefore, employing a water-soluble polymer, like poly (ethylene glycol) (PEG) and polysaccharides, to switch the polymer/siRNA complex has been a serious strategy to decrease such non-specific interactions, and thereby prolong the blood circulation time. Sato et al. studied a cationic comb-type copolymer (CCC) consisting of a polycation backbone and side chains of water-soluble polymer and located that the dense brush of the water-soluble side-chain polymer enhanced inter-polyelectrolyte complex between the polycation backbone

and siRNA. Furthermore, the CCC/siRNA complex exhibited a protective effect against nuclease activity and produced prolonged circulation time of siRNA in mice.

To avoid the drawbacks of cationic lipids like immunogenicity and instability within the serum, neutral liposomal delivery systems have also been developed for siRNA delivery. Halder et al. successfully delivered siRNA against FAK into human ovarian tumor in nude mice by neutral liposome 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC). The siRNA formulation inhibited FAK expression for up to 4 days in tumor tissue and reduced mean tumor weight by 44-72% in three different human ovarian cell lines (HeyA8, A2780-CP20 and SKOV3ip1). This group also reported recently that siRNA targeting IL-8 incorporated into neutral liposomes (siRNA-DOPCs) reduced the mean tumor weight by 32% and 52% within the HeyA8 and SKOV3ip1 mouse models and also decreased the micro vessel density of those tumors. Since the liposomes didn't contain charges, it's unclear how siRNA might be efficiently encapsulated within the liposomes.

B. Antibodies and Proteins: The recently developed systemically administered, tumor-specific immunoliposome nanocomplex with high transfection efficiency could function as a carrier for siRNA delivery and be utilized as effective anti-cancer clinical modalities when formulated with a therapeutic siRNA.

A receptor-specific antibody delivery system and avidin - biotin technology was used to deliver siRNA into a brain tumor across the blood - brain barrier. The siRNA was mono-biotinylated on either terminus of the sense strand, in parallel with the assembly of a conjugate of the targeting antibody and streptavidin. Intravenous administration of the transferrin receptor antibody attached siRNA caused 69-81% suppression in luciferase reporter gene expression within the intracranial brain cancer (C6 or RG-2) model in vivo. This study showed a therapeutic potential for brain cancer gene therapy.

Pirollo et al. have developed an antitransferrin receptor (TfR) single-chain antibody fragment-directed nanoimmunoliposome to deliver siRNA to both primary tumor and metastatic disease. A pH-sensitive histidine - lysine peptide and a modified hybrid (DNA-RNA) anti-HER-2 siRNA molecule were used to enhance the efficiency of this complex. The nanoimmunoliposome anti-HER-2 siRNA complex could silence the target gene and its downstream pathway components in vivo, sensitize the tumor cells to chemotherapeutic agents and inhibit tumor growth during a carcinoma model. Triche et al. have designed a non-viral

delivery system employing a cyclodextrin-containing polycation to bind and protect siRNA and transferrin. The targeted non-viral delivery system which systematically delivers siRNA against the EWS-FLI1 gene can inhibit tumor growth during a murine model of metastatic Ewing's sarcoma.

A polymer, OEI-HD conjugated with transferrin, was used for siRNA transfer into the mouse Neuro2a neuroblastoma cells in vitro and in vivo. siRNA against the Ras-related nuclear protein Ran delivered by transferrin-conjugated OEI-HD (three i.v. applications at 3-day interval) resulted in >80% reduced Ran protein expression, increased apoptosis and a reduced tumor growth during a xenograft Neuro2A tumor models without unspecific toxicity. Both non-targeted and transferrin-targeted siRNA nanoparticles showed similar biodistribution and tumor localization through the improved

permeability and retention (EPR) effect. However, the transferrin-targeted siRNA nanoparticles decreased luciferase activity within the tumor quite the non-targeted nanoparticles. Their results demonstrated that the function of the targeting ligand is to reinforce the cellular uptake in tumor cells instead of tumor localization. Li et al. reached an equivalent conclusion in their work using the anisamide ligand.

Conclusion: We considered the survivin siRNA with Cy3 fluorescence and injection to nude mice with MHCC-97H cells, and located intense Cy3 delivery in tumor mass, spleen and liver, but scant delivery in heart, bone marrow, brain, lung, and alimentary canal. Our study revealed that survivin siRNA nanoparticles were capable of inhibiting tumor growth both in vitro and in vivo.

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