

DOCTORAL THESIS

A novel nucleolin aptamer-celastrol conjugate (NACC) with super antitumor activity on advanced pancreatic cancer

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Abstract

Advanced pancreatic cancer (APC) has a poor prognosis due to the high degree of resistance after systemic chemotherapy. Celastrol (CSL), a quinone methyl triterpenoid monomer extracted from *Tripterygium wilfordii Hook F*, exhibits superior antitumor activity on pancreatic cancer (PC) both *in vitro* and *in vivo*. In addition, CSL counteracts multiple mechanisms involved in multi-drug resistance (MDR) of PC cells. However, CSL induced toxicity to normal tissues (e.g. liver) is the major impediment to its clinical application. Thus, it is desirable to seek strategy to facilitate CSL selectively targeting PC tissues and simultaneously reducing its exposure to healthy tissues (e.g. liver).

Aptamers are single-stranded oligonucleotides which specifically recognize and bind to targets by distinct secondary or tertiary structures. Nucleolin, a protein overexpressed on the plasma membrane of PC cells than that of normal cells (e.g. liver cell), which shuttle between cell surface, cytoplasm and nucleus, work as a cell surface receptor. Nucleolin aptamer is an anti-proliferative G-rich oligonucleotide with high affinity and specificity to nucleolin, which has been proved to be safe in clinical research. Then, nucleolin aptamer, as a target moiety, provide an approach to facilitate CSL selectively targeting PC cells. Taken together, our hypothesis is that the nucleolin aptamer modification could facilitate the conjugated CSL selectively targeting pancreatic cancer cells to achieve higher antitumor activity and less liver toxicity.

In our study, CSL was conjugated to nucleolin aptamer to form Nucleolin Aptamer-Celastrol Conjugate (NACC). A CRO Aptamer-Celastrol conjugate (CACC) was also synthesized as a control for comparison. The water solubility of NACC was significantly higher than that of CSL. Then, the molecular weight of NACC was detected by ESI mass spectrum (MS). The anti-proliferative efficacy of NACC was higher than CSL *in vitro*. NACC could selectively bind to PANC-1 cells over normal liver cells. The cellular uptake of NACC by PANC-1 cell was stronger than CSL. Moreover, NACC could be taken up by PANC-1 cells mainly via macropinocytosis. Tissue distribution study revealed that NACC could selectively accumulate in pancreatic tumor tissue and reduce the distribution in liver *in vivo*. In addition, NACC demonstrated higher antitumor activity and less liver toxicity *in vivo*, compared with CSL and CACC.

The above results revealed that the nucleolin aptamer modification could facilitate the conjugated CSL selectively targeting PC cells to achieve higher antitumor activity and less liver toxicity.

Table of Contents

DECLARATION.....	i
Abstract.....	ii
Acknowledgement.....	iv
Table of Contents	vi
Lists of Figures and Tables	x
List of Abbreviation.....	xi
Chapter 1 Background.....	1
1.1 The epidemiology of pancreatic cancer and the major challenges.....	1
1.2 The causes of chemotherapy tolerance in pancreatic cancer and the related molecular mechanisms.....	3
1.2.1 P-glycoprotein and MDR in pancreatic cancer.....	4
1.2.2 NF-kB pathway and MDR in pancreatic cancer.....	7
1.2.3 HSP90 and MDR in pancreatic cancer.....	9
1.2.4 Tumor angiogenesis and MDR in pancreatic cancer.....	12
1.3 Celastrol as a potential drug for treatment of pancreatic cancer and the impediment to its clinical application.....	15
1.4 The application of aptamer in tumor targeted therapy.....	18
1.5 Nucleolin aptamer as a moiety to target pancreatic cancer.....	21
1.6 Research Hypothesis.....	22
Chapter 2 Materials and Methods	24
2.1 Materials for chemical synthesis	24
2.2 Preparative high performance liquid chromatography (Pre-HPLC) analysis for purifying compounds.....	25
2.3 Nuclear magnetic resonance (NMR) analysis for indentifying compounds	25
2.4 Reversed-phase high performance liquid chromatography (RP-HPLC) analysis for indentifying compounds.....	26

2.5 Cell culture	26
2.6 Cell proliferation study	27
2.7 Animal handling.....	27
2.8 Xenograft tumor model of pancreatic cancer	28
2.9 Flow cytometry.....	28
2.10 Cellular uptake pathways	29
2.11 <i>In vivo</i> injection of Microfil and Micro CT.....	30
2.12 Western blot analysis.....	30
2.13 Tissues and cellular distribution	31
2.14 Blood biochemical analysis.....	32
2.15 Histological analysis	32
2.16 Statistical analysis.....	32
Chapter 3 The effect of modification on the carboxylic acid group on the antitumor activity of celastrol.....	33
3.1 Aim.....	33
3.2 Design	33
3.3 Results.....	34
Chapter 4 The synthesis and characterization of Nucleolin Aptamer-Celastrol Conjugate (NACC).....	36
4.1 Aim.....	36
4.2 Synthesis of the celastrol derivative	36
4.2.1 Experimental design.....	36
4.2.2 Results	37
4.3 Synthesis process of Nucleolin Aptamer-Celastrol Conjugate (NACC). 38	
4.3.1 Experimental design.....	38
4.3.2 Results	39
4.4 Synthesis process of the CRO Aptamer-Celastrol Conjugate (CACC) ...	40
4.4.1 Experimental design.....	40
4.4.2 Results	41
4.5 The water solubility and the stability of the NACC <i>in vitro</i>	42

4.5.1 Experimental design.....	42
4.5.2 Result.....	42
Chapter 5 The effect of the nucleolin aptamer modification on the cytotoxicity of the conjugated CSL in NACC <i>in vitro</i>	44
5.1 Aim.....	44
5.2 Experimental design.....	44
5.3 Results.....	44
Chapter 6 The effect of the nucleolin aptamer modification on the cellular uptake and the cellular internalization of NACC <i>in vitro</i>	46
6.1 Aim.....	46
6.1.1 To investigate the selectivity of NACC to pancreatic cells and the cellular uptake <i>in vitro</i>	46
6.1.2 To investigate the mechanisms of cellular uptake of NACC by pancreatic cancer cells <i>in vitro</i>	46
6.2 Experimental design.....	46
6.3 Results.....	47
Chapter 7 The effect of the nucleolin aptamer modification on the distribution of NACC in a xenograft mouse model of human pancreatic cancer.....	52
7.1 Aim.....	52
7.2 Experimental design.....	52
7.3 Results.....	53
Chapter 8 The effect of the nucleolin aptamer modification on the antitumor activity of the NACC in a xenograft mouse model of human pancreatic cancer ..	57
8.1 Aim.....	57
8.1.1 To investigate the antitumor efficacy of NACC in xenograft tumor model of pancreatic cancer.	57
8.1.2 To investigate the effect of NACC on angiogenesis of tumor microenvironment.....	57
8.1.3 To investigate the effect of NACC on expression level of NF- κ B, HSP 90 and P-glycoprotein in tumor tissue.	57

8.1.4 To investigate the effect of NACC on survival of tumor bearing mice.....	57
8.2 Experimental design.....	57
8.3 Results.....	59
Chapter 9 The effect of the nucleolin aptamer modification on the liver toxicity of NACC.....	66
9.1 Aim.....	66
9.2 Experimental design.....	66
9.3 Result	67
Chapter 10 Discussion.....	70
References.....	76
CURRICULUM VITAE.....	96