ABSTRACT

According to the cancer stem cells (CSCs) hypothesis, CSCs are responsible for the treatment failures. CSCs are a subset of cells possessing stemness properties within the heterogeneous tumor mass. Therapeutic intervention on Wnt signaling is of our great interest because an aberrant Wnt signaling is an important driver to maintain the potency of CSCs. In nasopharyngeal carcinoma (NPC), deregulated expression of the Wnt signaling components is frequently observed. ICG-001 is a selective Wnt modulator (CBP antagonist) that specifically interrupts the interaction between β-catenin and CBP, thereby encourages the interaction between β-catenin and p300 and the subsequent differentiation and reduction of the CSCs subset. For this reason, the present study aimed to evaluate the therapeutic potential of ICG-001 in NPC.

Results showed that ICG-001 inhibited both the migration of the NPC cells and the formation of tumor spheres. In the first part of the mechanistic studies (Chapter 3), ICG-001 was found to restore the expression of miR-150 in NPC cells. MiR-150 was further found to directly reduce CD44 expression and inhibit NPC cell migration. In the second part of the mechanistic studies (Chapter 4), ICG-001 was found to reduce the expression of Evi1 in NPC cells. The effect was accompanied with the inhibition of both the NPC cells migration and the tumor spheres formation. Two molecular axes, namely miR-96/Evi1/miR-449a and survivin/Evi1/miR-449a, were found to be involved in the inhibition of the tumor cell migration and spheroids formation. The therapeutic potential of using this CBP antagonist (ICG-001) in NPC, namely the in vitro and in vivo efficacy of ICG-001 combined with cisplatin, was examined (Chapter 5). Concurrent treatment of ICG-001 and cisplatin exhibited a synergistic inhibition on the in vitro growth and the tumor sphere forming capacity of NPC cells as well as the growth of NPC xenografts. Taken together, results presented in this thesis suggested that ICG-001 (PRI-724 is the analog of ICG-001 currently used in clinical trials) has a therapeutic potential in NPC.
# TABLE OF CONTENTS

Declaration i  
Abstract ii  
Acknowledgements iii  
Table of Contents v  
List of Tables xi  
List of Figures xii  
List of Abbreviations xvi

## Chapter 1  Introduction  
1.1 Nasopharyngeal carcinoma (NPC) 2  
  1.1.1 Epidemiology 2  
  1.1.2 Histological classification 4  
  1.1.3 Diagnosis and staging 4  
  1.1.4 Etiology 8  
  1.1.5 Treatments 9  
  1.1.6 Wnt signaling in NPC tumorigenesis 10  
1.2 Cancer stem cells (CSCs) 12  
  1.2.1 Hypothesis 12  
  1.2.2 Definition and properties 14  
  1.2.3 CSCs in NPC 15  
  1.2.4 Wnt/β-catenin and therapeutic implication 16  
1.3 MicroRNAs (miRNAs) 18  
  1.3.1 Canonical biogenesis pathway 18
1.3.2 Cellular functions

1.4 CBP antagonists: ICG-001 and PRI-724

1.4.1 Molecular mechanisms of action

1.4.2 Progress of clinical trials using CBP antagonists in cancer therapy

1.5 Scopes of the study

Chapter 2  Materials and Methods

2.1 Materials

2.2 Cell culture

2.3 Plasmid constructs

2.4 Cell growth assay

2.5 Transient transfection

2.6 Transwell migration assay

2.7 Tumor spheroid formation assay

2.8 Western blotting

2.9 miRNA target prediction

2.10 Luciferase reporter assay

2.10.1 TCF/LEF reporter assay

2.10.2 miRNA target analysis

2.11 Quantitative real-time PCR (qRT-PCR)

2.11.1 Total RNA extraction

2.11.2 mRNA expression analysis

2.11.3 miRNA expression analysis

2.12 Co-Immunoprecipitation
### 2.13 Nude mice tumorigenicity assay

2.13.1 Housing 34

2.13.2 Surgery 34

2.13.3 NPC xeno-2117 xenograft study 34

2.13.4 NPC C17 xenograft study 35

### 2.14 Drug combination studies 36

### 2.15 Statistical analysis 37

### Chapter 3  CD44 is involved in the anti-migratory effect of ICG-001 on NPC 38

#### 3.1 Introduction 39

#### 3.2 Results 41

3.2.1 Effect of ICG-001 on the growth of NPC cells 41

3.2.2 ICG-001 inhibits canonical Wnt signaling in NPC cells 41

3.2.3 ICG-001 inhibits the migration of NPC cells 42

3.2.4 Knockdown of β-catenin or CBP inhibits the migration of C666-1 cells 42

3.2.5 ICG-001 down-regulates the expression of CD44 42

3.2.6 Knockdown of CD44 inhibits the migration of C666-1 cells but not the growth of tumor spheres 43

3.2.7 Association between ezrin and CD44 in C666-1 cells 43

3.2.8 ICG-001 enhances the expression of miR-150 44

3.2.9 CD44 is a novel target of miR-150 44

3.2.10 Overexpression of miR-150 resulted in the reduction of the migration of C666-1 cells but not the growth 45
Chapter 4  Evi1 is involved in the growth suppression and migration of NPC cells
4.2.10 Effect of silencing or overexpression of Evi1 on the expression of miR-96 and miR-449a 84
4.2.11 The coordination between miR-96 and miR-449a 84
4.2.12 Knockdown of β-catenin or CBP inhibits tumor spheroid formation 85
4.2.13 Knockdown of β-catenin or CBP enhances the expression of miR-96 and miR-449a 85
4.2.14 Knockdown of p300 reduces the expression of miR96 and miR449a 85
4.2.15 ICG-001 reduces the protein expression of survivin 86
4.2.16 Silencing of survivin represses the expression of Evi1 86
4.2.17 Effect of survivin knockdown on the expression of miR-96 and miR-449a 87

4.3 Discussion 88

Chapter 5 ICG-001 enhances the treatment efficacy of cisplatin on NPC 128

5.1 Introduction 129
5.2 Results 130
5.2.1 Effect of ICG-001/cisplatin concurrent therapy on the growth of NPC cells 130
5.2.2 Effect of ICG-001/cisplatin concurrent therapy on tumor spheroid formation 131
5.2.3 Effect of ICG-001/cisplatin concurrent therapy in nude mice tumorigenicity assay 131