# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Tables</td>
<td>i</td>
</tr>
<tr>
<td>List of Figures</td>
<td>ii-iii</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>iv-v</td>
</tr>
<tr>
<td>Abstract</td>
<td>vi-vii</td>
</tr>
<tr>
<td>Preface</td>
<td>viii-x</td>
</tr>
<tr>
<td>Chapter 1: A case for NM23H2 in tumor metastasis</td>
<td>1-27</td>
</tr>
<tr>
<td>1.1 Tumor metastasis is a complex process</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Winning battle against metastasis will eventually lead to better</td>
<td>5</td>
</tr>
<tr>
<td>cancer cure</td>
<td></td>
</tr>
<tr>
<td>1.3 Clinical implications of breast cancer metastasis</td>
<td>6</td>
</tr>
<tr>
<td>1.4 Genetic aspects of metastasis: role of metastasis suppressor genes</td>
<td>8</td>
</tr>
<tr>
<td>1.5 Metastasis suppressor genes are diverse in nature</td>
<td>9</td>
</tr>
<tr>
<td>1.6 Targeting metastasis suppressor genes for halting cancer progression</td>
<td>11</td>
</tr>
<tr>
<td>1.7 Discovery of NM23 gene family</td>
<td>13</td>
</tr>
<tr>
<td>1.8 An introduction to NM23H2 gene and its regulation</td>
<td>14</td>
</tr>
<tr>
<td>1.8.1 NM23H2 gene, mRNA and transcripts</td>
<td>14</td>
</tr>
<tr>
<td>1.8.2 NM23H2 protein, structure and localization in the cell</td>
<td>14</td>
</tr>
<tr>
<td>1.8.3 Regulation of NM23H2 transcript and protein</td>
<td>15</td>
</tr>
<tr>
<td>1.9 NM23H2 is a multi-functional protein</td>
<td>16</td>
</tr>
<tr>
<td>1.9.1 Enzymatic roles of NM23H2</td>
<td>17</td>
</tr>
<tr>
<td>1.9.1.1 NM23H2 as a nucleoside diphosphate kinase</td>
<td>17</td>
</tr>
<tr>
<td>1.9.1.2 NM23H2 as a protein histidine kinase</td>
<td>18</td>
</tr>
<tr>
<td>1.9.2 Functions of NM23H2 through protein- protein interactions</td>
<td>19</td>
</tr>
<tr>
<td>1.9.2.1 NM23H2 induces desmosomes formation</td>
<td>20</td>
</tr>
<tr>
<td>1.9.2.2 NM23H2 induces apoptosis</td>
<td>20</td>
</tr>
<tr>
<td>1.9.2.3 NM23H2 mediated control of cell migration and invasion</td>
<td>20</td>
</tr>
<tr>
<td>1.9.2.4 NM23H2 mediated endocytosis of a G protein coupled receptor (GPCR)</td>
<td>21</td>
</tr>
</tbody>
</table>
1.9.2.5 NM23H2 mediated vesicle formation through coat protein complex II assembly in mammalian cells

1.9.2.6 NM23H2 is associated with shelterin complex proteins at telomeres

1.9.3 Functions of NM23H2 through DNA binding: Role in gene regulation

1.9.3.1 Role of NM23H2 in gene regulation as a transcription factor

1.9.3.2 NM23H2 acting as transcriptional co-factor

1.10 NM23H2 is a metastasis suppressor gene across tumor types even though mechanisms are poorly understood

1.11 A strategy to investigate novel roles of NM23H2 in breast cancer metastasis

Chapter 2: NM23H2 as a key metastasis suppressor in breast cancer

2.1 Introduction

2.1.1 Examination of previous reports of NM23H2 acting as metastasis suppressor in breast cancer

2.1.2 A combinatorial strategy for assessing relationship of NM23H2 to patient survival, and regulation of metastasis in vitro and in vivo

2.2 Results

2.2.1 Meta-analysis of >30 MSGs shows consistently reduced expression of NM23H2 in advanced breast tumors

2.2.2 Higher NM23H2 level within primary tumors associated with enhanced overall patient survival

2.2.3 NM23H2 levels are decreased in advanced breast tumors; qPCR analysis of patient tumors

2.2.4 NM23H2 expression is reduced in autologous lymph node metastasis

2.2.5 Differential expression of NM23H2 in breast cancer cell lines that differ in metastatic potential

2.2.6 Generation and validation of breast cancer cells with stably increased expression of NM23H2

2.2.7 NM23H2 over-expressing cells have reduced invasive potential through basement membrane matrix

2.2.8 NM23H2 over-expressing cells have reduced trans-endothelial migration potential

2.2.9 NM23H2 suppresses metastasis in vivo
2.3 Discussion
   2.3.1 Integrative approaches support a role for NM23H2 in breast tumor metastasis suppression 42
   2.3.2 A case for gene expression regulatory role of NM23H2 in control of breast cancer metastasis 42

2.4 Materials and Methods
   2.4.1 Cells and culture conditions 43
   2.4.2 Western Blotting 43
   2.4.3 Meta-analyses of patient samples 44
   2.4.4 Survival analysis of patient samples 44
   2.4.5 Generation of MDMB-231 cells stably over expressing for NM23H2 protein 44
   2.4.6 Cell invasion and trans-endothelial migration assays 45
   2.4.7 Clinical annotation of tumor specimen analyzed 45
   2.4.8 Immunohistochemical analysis 46
   2.4.9 Tail vein metastases assay 46
   2.4.10 Statistical analysis 46

Chapter 3: Gene expression profiling of NM23H2 over expressing MDA-MB-231 breast cancer cells suggest control of biological pathways crucial for metastasis 47-59

3.1 Introduction
   3.1.1 Gene expression microarrays have been used as important prognostic and predictive tools for tumor progression in several cancer types 47
   3.1.2 A case for global gene expression profiling of NM23H2 over expressing MDA-MB-231 breast cancer cells 47

3.2 Results
   3.2.1 Gene expression profiling upon over expression of NM23H2 suggested control of multiple signaling pathways 49
   3.2.2 Comparison of NM23H2 over expressing MDA-MB-231 transcriptome with NM23H2 over expressing CAL27 transcriptome 51
   3.2.3 Contribution of NM23H2 target genes to clinical progression of breast cancer 52
   3.2.4 Validation of genome wide expression microarray by high throughput EMT specific qPCR assay 54

3.3 Discussion 55
3.3.1 NM23H2 over expression in MDA-MB-231 cells led to profound changes in gene expression  

3.3.2 Clues for regulation of EMT and MET from gene expression profile  

3.4 Materials and Methods  

3.4.1 Cells and culture conditions  

3.4.2 Gene expression profiling in NM23H2 over expressing MDA-MB-231 breast cancer cells  

3.4.3 Gene ontology and pathway analysis  

3.4.4 Meta-analysis of gene expression related to comparison between CAL27 and MDA-MB-231 cells  

3.4.5 Meta-analysis of gene expression with tumor sample transcriptome  

3.4.6 EMT qPCR array  

3.4.7 Statistical analysis  

Chapter 4: NM23H2 regulates metastasis by opposing TGF beta mediated EMT in breast cancer cells  

4.1 Introduction  

4.1.1 A case of exploring TGF beta signaling by NM23H2  

4.1.2 Role of TGF beta pathway in EMT regulation  

4.2 Results  

4.2.1 NM23H2 over expressing MDA-MB-231 breast cancer cells has distinct changes in morphology and molecular markers of EMT  

4.2.2 TGFBR2 expression is down regulated in NM23H2 over expressing MDA-MB-231 breast cancer cells  

4.2.3 Relationship between NM23H2 and TGFBR2 expression in MDA-MB-468 breast cancer cells  

4.2.4 NM23H2 physically localizes on TGFBR2 promoter  

4.2.5 Mechanisms of transcriptional repression of TGFBR2 promoter by NM23H2: loss of activating histone marks and enhanced recruitment of histone demethylase, LSD1 to TGFBR2 promoter  

4.2.6 NM23H2 physically interacts with LSD1  

4.2.7 NM23H2 opposes TGF beta signaling in breast cancer cells  

4.2.8 Diminished phosphorylation in SMAD3 levels in NM23H2 over expressing MDA-MB-231 breast cancer cells
### Chapter 4

4.2.9 ChIP assay revealed altered SMAD4 localization on E-Cadherin promoter

4.2.10 NM23H2 regulates EMT through targeting TGFBR2

4.2.11 NM23H2 and TGFBR2 expression levels are inversely correlated in clinical samples of breast cancer

4.2.12 How is NM23H2 reduced in expression during metastasis progression: promoter hypermethylation?

### 4.3 Discussion

4.3.1 NM23H2 and regulation of TGF beta signaling

4.3.2 Control of EMT-MET by NM23H2

4.3.3 NM23H2 as a key metastasis suppressor gene in breast cancer

### 4.4 Limitations and merits of the study

4.4.1 Limitations of present study

4.4.2 Take home message of thesis: NM23H2 dependent control of EMT through TGF beta signaling as key to breast cancer metastasis

### 4.5 Materials and Methods

4.5.1 Cells, culture conditions, microscopy and invasion assays

4.5.2 RNA isolation, cDNA and qPCR

4.5.3 Western blotting and co-immunoprecipitation

4.5.4 ChIP assay

4.5.5 Luciferase Reporter Assay

4.5.6 Promoter Methylation Analysis

4.5.7 5-Aza-2'-deoxycytidine treatment and expression analysis of NM23H2 in MDA-MB-231 cells

4.5.8 Meta-analysis for relationship between gene expression and patient survival

4.5.9 Statistical analysis

### Chapter 5: Summary and Conclusions

### Bibliography

### Appendix

### List of Publications