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## Unraveling multifaceted roles of Grainyhead-like transcription factor-2 in breast cancer

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# Chapter 1

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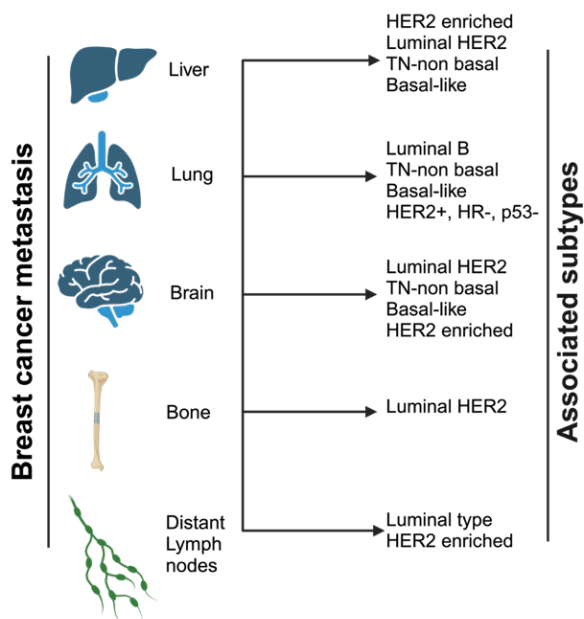
Introduction, aim and scope of  
the thesis

### Breast Cancer

Breast cancer is the leading cause of death among women worldwide.<sup>1</sup> The highly heterogeneous and complex nature of the disease, characterized by diverse biological and histological features influence the breast cancer prognosis and response to the treatments. To overcome this heterogeneity and tailor the treatment options, breast cancer has been subcategorized into four groups based on the expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2); Luminal A, Luminal B, Human epidermal growth factor receptor 2 enriched (HER2<sup>+</sup>) and Triple-negative Basal-like.<sup>2</sup>

Luminal A tumors accounts for the most prevalent subtype with ER, PR and low Ki67 expression (a proliferation marker) but not HER2. It shows good prognosis and hormone therapy response. Compared to Luminal A, Luminal B has a higher level of Ki67 expression, causing a worse prognosis. It represents 20% of luminal tumors and expresses ER, PR and Her2. The Her2 enriched subtype is characterized by overexpression of Her2 and absence of ER and PR. HER2<sup>+</sup> breast tumors are associated with poor prognosis and require therapies targeting Her2 such as trastuzumab.<sup>3,4</sup> The last subtype Basal-like lacks ER, PR and Her2 expression. The high metastatic potential and the aggressiveness of TNBC tumors result in poor prognosis and survival outcomes.<sup>5,6</sup>

While this categorization based on the molecular markers guides the disease prognosis and treatment strategies, metastasis remains a major challenge for achieving therapeutic success. Metastasis accounts for more than 90% of cancer related deaths. It has been previously reported that the interactions between the components of tumor microenvironment (TME) (extracellular matrix, tumor cells, fibroblasts, blood and lymph vessels, immune cells and cytokines) promotes tumor progression and metastasis.<sup>7-10</sup> Therefore, it is crucial to identify key molecules and processes modulating the TME to channel novel targeted therapies to combat metastasis.



**Figure 1: Association of breast cancer molecular subtypes with metastasis.**

Organ-specific metastasis of breast cancer is governed by the molecular subtypes of breast cancer. The most prevalent metastatic site in breast cancer patients is the bone, followed by the brain as the second most common site, with the liver and lungs being subsequent sites. (Created by Biorender.com)

## Grainyhead-like transcription factors

Transcriptional regulatory networks play pivotal roles in embryogenesis, wound healing and disease-onset (e.g., cancer) in humans. The Grainyhead-like (Grhl) family of transcription factors belong to such group of genes. It was initially identified in *Drosophila melanogaster* which harbor an embryonic mutation, resulting in a unique head defect phenotype characterized by holes in specific large cuticular regions and abnormal cuticular structures.<sup>11</sup> Three orthologues of Grhl; GRHL1-3 are expressed in mammals.<sup>12,13</sup> GRHL transcription factors consist of an N-terminal transactivation domain, a DNA binding domain homologous to tumor suppressor gene p53, a DNA binding domain, and a C-terminal dimerization domain, enabling GRHL proteins to form homo and heterodimers.<sup>14</sup>

GRHL family members exert tissue-specific functions during mammalian development by being involved in the regulation of neural tube closure,<sup>15,16</sup> formation of the lungs,<sup>17,18</sup> skin barrier function,<sup>19,20</sup> and epithelial morphogenesis.<sup>21</sup> They are also involved in the repair of epidermal barrier after tissue damage modulated by receptor tyrosine kinases.<sup>22</sup> These important roles are facilitated in part by the negative regulation of epithelial-mesenchymal transition (EMT); a cellular process where epithelial cells lose apical-basal polarity and cell-cell adhesion through downregulation of epithelial genes<sup>23,24</sup> (i.e. E-cadherin, OVOL2) enabling to gain mesenchymal features such as motility, invasion and plasticity through upregulation of mesenchymal genes (i.e.; ZEB1, Vimentin SNAIL1).<sup>25</sup> EMT in embryonic development also lead to the differentiation of germ layers (ectoderm, endoderm and mesoderm), giving rise to the formation of different organs.<sup>26</sup> The members of GRHL family act as master regulators of epithelial characteristics and drive the establishment and maintenance of tissue integrity and epithelial differentiation in the embryonic development and tissue homeostasis.

In addition to their roles in the developmental processes, GRHL family transcription factors have been linked to various types of cancer including breast cancer,<sup>23,27</sup> lung cancer,<sup>28,29</sup> ovarian cancer,<sup>30</sup> colorectal cancer (CRC),<sup>31,32</sup> skin cancer,<sup>33,34</sup> and neuroblastoma.<sup>35</sup> This association is characterized by the disruption of the epithelial integrity and the dysregulation of growth and survival pathways, exhibiting both tumor suppressive and supportive functions, in part through modulation of EMT.<sup>36–38</sup>

### **Deregulation of GRHL1 and GRHL3 in cancer**

GRHL1 has been associated with squamous cell carcinoma of the skin. *Grlh1* deletion in mice resulted in skin barrier defects, accompanied by more chemically-induced skin tumor formations.<sup>33</sup> This implied that GRHL1 functions as a tumor suppressor gene. Another significant role of GRHL1 as tumor suppressor has been reported in neuroblastoma. GRHL1 was identified as an early response gene to the treatment with histone deacetylase inhibitors. Its overexpression in neuroblastoma cell lines inhibited proliferation and hampered anchorage independent growth. Neuroblastoma patients with high

GRHL1 expression had favorable prognosis.<sup>35</sup> In contrast, a tumor promoter function of GRHL1 in cell-cycle regulation via EGFR-ERK axis has been shown in lung cancer.<sup>28</sup>

Similar to GRHL1, GRHL3 also exhibits context-specific activities in multiple tumors types. GRHL3 was found to be expressed mainly in early stage and hormone receptor positive breast cancers and decreased GRHL3 expression was observed during tumor progression. This indicated a possible function of GRHL3 in suppressing the tumor growth.<sup>39</sup> However, an overexpression study of GRHL3 in skin and breast cancer cell lines showed the direct transcriptional repression of E-cadherin by GRHL3, resulting in induction of cell migration and invasion.<sup>40</sup> Furthermore, decreased GRHL3 expression levels promoted EMT and deregulated MAPK/ERK pathway in CRC cells, demonstrating the tumor promoter role of GRHL3.<sup>41</sup> In addition to CRC, reduced levels of GRHL3 in squamous cell carcinoma of the skin also contributed to the tumor progression and upregulated of the oncomir miR-21, an indicator of malignant transformation.<sup>42</sup>

### **GRHL2 transcription factor in tumorigenesis**

GRHL2 is located on the chromosome 8q22 within human genome, a region that is commonly amplified in different tumor types.<sup>43</sup> Dysregulation of GRHL2 expression has been linked to numerous cellular processes, affecting the tumor progression and metastasis. In this regard, GRHL2 confers both tumor suppressive<sup>44,45</sup> and oncogenic roles in cancers.<sup>46,47</sup>

#### *GRHL2 as a tumor suppressor*

It has been demonstrated that the overexpression of GRHL2 expression prevented the invasive and migratory capabilities of gastric cancer cell lines and transforming growth factor- $\beta$  (TGF $\beta$ )-induced EMT in both gastric cancer<sup>48</sup> and oral carcinogenesis.<sup>49</sup> Downregulation of GRHL2 expression in lung cancer cell lines induced partial EMT -a hybrid state where the expression of both epithelial and mesenchymal genes coexists- by influencing the cell proliferation and colony formation.<sup>29</sup> Moreover, functional studies on GRHL2 expression revealed that GRHL2 inhibits EMT through its direct interactions with E-



to the promoters of RAB25, CLDN4, ARHGEF19, and ERBB as well as intron of E-cadherin/CDH1, resulting in alterations in histone methylation. GRHL2 also inhibits SNAIL2, TWIST1 and TGF-beta/Wnt-induced EMT. Furthermore, it increases ICAM-1 expression and the sensitivity of NK killing via KMT2C/D interactions and inhibition of p300. GRHL2 is downregulated by miR-133a, resulting in decreased ESRP1 expression and EMT inhibition. MiR-762 reduction by circTNRC18 increases GRHL2 expression. EMT enhances mitochondrial oxidative phosphorylation accompanied by the overall declined level of ROS and increased GLUD1 expression which is restored by GRHL2. EMT elevates mitochondrial oxidative phosphorylation, leading to a decrease in ROS levels and an increase in GLUD1 expression. This effect is reversed by GRHL2. (Adopted from He J. et.al.; 2020)

### *GRHL2 as an oncoprotein*

Several studies have also attributed a tumor supportive role for GRHL2. A study with colorectal cancer cell lines identified GRHL2 as an oncoprotein due to its ability for enhancing cell viability and decreasing apoptosis through PI3K/Akt pathway.<sup>31</sup> Increased GRHL2 expression has been associated with poor prognosis and resistance to cisplatin, regulated by via ERK/MAPK signaling in ovarian cancer.<sup>53</sup> GRHL2 also employed oncogenic roles in lung cancer by stabilizing partial EMT; a hybrid epithelial/mesenchymal phenotype that holds a higher metastatic potential than the mesenchymal state.<sup>24</sup> Lastly, it has been found that GRHL2 functions as a key player for telomerase activity by controlling hTERT expression, thereby enhancing the viability of tumor cells.<sup>54</sup>

### *Role of GRHL2 in stemness and epigenetic regulation*

A mechanism based mathematical modeling analyzed the tumor initiating potential (stemness) of GRHL2 in stemness through EMT. GRHL2-centered positive and negative feedback loops including ZEB1 and SNAIL constituted a regulatory network between GRHL2 and stemness associated genes such as Oct4 and LIN28.<sup>24</sup> Direct regulation of Oct4 by GRHL2 modulated the stemness and plasticity in oral cancer cells.<sup>47</sup> Notably, decreased GRHL2 expression enabled pancreatic cancer cells to retain their stem-like characteristics, including self-renewal capacity and resistance to anoikis.<sup>45</sup>



Multiple studies have examined the interaction between GRHL2 and epigenetic modifiers, particularly Histone deacetylases (HDACs). HDACs are enzymes that induce loss of gene expression through chromatin condensation, impacting cellular processes such as cancer stemness, cell proliferation, and EMT.<sup>55</sup> The induction of GRHL2 suppresses the expression of mesenchymal proteins like ZEB1 and SNAI2 and augments apoptosis mediated by HDAC inhibitors in glioblastoma.<sup>56</sup> In a study on Basal-like breast cancer, the mechanisms behind cell survival upon treatment with conventional therapy were linked to the loss of histone acetylation by H3K27ac, a known transcription enhancer, at regulatory regions of epithelial master regulators like GRHL2.<sup>57</sup>

### **GRHL2 in breast cancer**

GRHL2 exhibits context-specific and subtype-specific functions in breast cancer, similar to its behavior in other cancer types. The role of GRHL2 in maintaining epithelial phenotype of breast cancer cells was highlighted above. This was further supported by Werner et al., who demonstrated that GRHL2 knockdown led to the downregulation of its target gene *ErbB3*, resulting in phenotypic and genotypic changes related to EMT in Basal subtype of breast cancer cells.<sup>58</sup> In contrast, elevated expression of GRHL2 has been associated with increased metastatic potential and poor relapse-free survival in breast cancer patients.<sup>59</sup>

Downregulated GRHL2 expression was observed in the Basal-B subtype.<sup>46</sup> Accumulating evidence suggested a specific role for GRHL2 in modulating transcriptional activity of ER in hormone receptor positive breast cancer. A virtual ChIP-seq based network analysis revealed that GRHL2 restricts estrogen transcriptional activity by downregulating the estrogen responsive enhancer RNAs.<sup>60</sup> In Tamoxifen-resistant breast tumors, FOXA1, a pioneer factor for ER transcriptional activity, has been discovered to interact with GRHL2. This interaction further regulated the LYPD3/AGR2 complex, promoting therapy resistance.<sup>61</sup> It was further shown that GRHL2 not only controls the ER activity but also prevents estrogen-induced migration of ER-positive breast cancer cells.<sup>62</sup> Altogether, these studies manifest a multifaceted role for GRHL2 function across multiple breast cancer subtypes.

### **Role of GRHL2 in immune modulation**

The immune landscape of the TME is heterogenous and determines tumor progression. Therefore, it is important to understand the dynamic interplay between tumor and immune cells.<sup>63</sup> The immune cells exert functional adaptations to the local TME and perform dual roles. These can be tumor growth promoting: Regulatory T cells (Treg), M2-like Tumor-associated Macrophages, Mast cells, Innate Lymphoid Cells type 2 and 3 (ILC2/3). Or they can be tumor growth inhibiting: Natural killer cells (NK) and Tumor infiltrating CD4+ and CD8+ Lymphocytes (TILs), Dendritic cells (DC).<sup>64</sup> During tumor progression, tumor cells adopt several immune evasive mechanisms to limit infiltration of anti-tumor immune cells and impair their cytotoxic or effector functions.<sup>65</sup> An overview of immune evasive mechanisms in breast cancer is shown in Figure 3.

Besides enabling the tumor cells to gain the cellular plasticity, EMT also confer immune evasion in different cancer types including breast cancer.<sup>66</sup> It has been previously reported that diverse epithelial and mesenchymal phenotypes modulate the sensitivity to the immune cells, thereby determining the therapy response in metastatic melanoma patients.<sup>67</sup> A comprehensive study on immune infiltration in breast cancer patient tumors revealed a correlation between EMT signature and the immune response by using the expression of epithelial markers associated with EMT. It has found out that in the triple-negative tumors and poorly differentiated tumors where E-cadherin and GRHL2 expression was downregulated, the inflammatory infiltrate was more prominent.<sup>68</sup> This was further supported by Song S. et.al, demonstrating the inverse correlation between GRHL2 expression and CD8+T cell infiltration in breast tumors.<sup>69</sup> Moreover, type I interferon (IRF1) has been identified as a direct target of GRHL2 and GRHL2 upregulated the expressions of both IRF1 and IRF3; stimulants of immune responses. Therefore, high GRHL2 protein expression was found to be associated with suppression of tumor recurrence.<sup>27</sup> Regulation of interferon response genes by GRHL2 has been also demonstrated in another study. Upon GRHL2 overexpression, an interaction of GRHL2 with two the histone methyltransferase genes was formed and this further induced mesenchymal-epithelial transition and sensitized the cells



binding sites as well as direct and indirect targets of GRHL2 in luminal breast cancer in chapter 3. ChIP-seq analysis revealed few overlapping binding sites of GRHL2 with ER $\alpha$  in luminal breast cancer cell lines. We found the direct and indirect targets of GRHL2 in response to GRHL2 loss in luminal breast cancer using Bru-seq. An integrative analysis of ChIP-seq and Bru-seq data identified distinct gene regulatory networks controlled by GRHL2 in luminal breast cancer, differ from those in other tissues. Chapter 4 demonstrates the shared and distinct roles for GRHL2 in growth and motility of luminal and basal breast cancers. A common outcome of GRHL2 deletion was characterized by cell cycle arrest. We observed a reduction in epithelial markers particularly in the luminal line while mesenchymal markers were induced only in basal cells alongside enhanced migration in response to GRHL2 loss. This is further confirmed by an in vivo model with silenced GRHL2 which demonstrated reduced primary tumor growth and fewer lung colonies, indicating that growth suppression predominated upon GRHL2 deletion. We focus on delineating GRHL2-mediated drug resistance/sensitivities in the Basal B subtype of breast cancer in Chapter 5. GRHL2 overexpression did not result in any MET-like changes in Basal B cell line in contrast to the literature previously reported. We also didn't find an effect of elevated GRHL2 expression on the cellular proliferation. A kinase inhibitor approach was implemented on GRHL2 overexpressing Basal B cells that four kinase candidates have been associated with the presence GRHL2. However, this needs further validations to confirm GRHL2 mediated responses. Chapter 6 focuses on the impact of GRHL2 deletion in immune modulation via NT5E/CD73 in luminal breast cancer. Loss of GRHL2 increased the expression of NT5E/CD73, resulting in elevated levels of adenosine production in the TME. This further enhanced the CD8 $^{+}$  T cell migration. In chapter 7, we provide an overview of the research presented in this thesis, accompanied by the discussions and future perspectives. Overall, this thesis unveils the novel regulatory roles of GRHL2 across breast cancer subtypes and highlighting novel GRHL2-mediated pathways for potential therapeutic interventions in breast cancer.

### References:

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209-249. doi:10.3322/caac.21660
2. Al-Thoubaity FK. Molecular classification of breast cancer: A retrospective cohort study. *Ann Med Surg Lond*. 2020;49:44-48. doi:10.1016/j.amsu.2019.11.021
3. Ellis MJ, Perou CM. The genomic landscape of breast cancer as a therapeutic roadmap. *Cancer Discov*. 2013;3(1):27-34. doi:10.1158/2159-8290.CD-12-0462
4. Orrantia-Borunda E, Anchondo-Nuñez P, Acuña-Aguilar LE, Gómez-Valles FO, Ramírez-Valdespino CA. Subtypes of Breast Cancer. In: Mayrovitz HN, ed. *Breast Cancer*. Exon Publications; 2022. Accessed May 20, 2024. <http://www.ncbi.nlm.nih.gov/books/NBK583808/>
5. Milioli HH, Tishchenko I, Riveros C, Berretta R, Moscato P. Basal-like breast cancer: molecular profiles, clinical features and survival outcomes. *BMC Med Genomics*. 2017;10(1):19. doi:10.1186/s12920-017-0250-9
6. Yin L, Duan JJ, Bian XW, Yu SC. Triple-negative breast cancer molecular subtyping and treatment progress. *Breast Cancer Res*. 2020;22(1):61. doi:10.1186/s13058-020-01296-5
7. Ferreira Almeida C, Correia-da-Silva G, Teixeira N, Amaral C. Influence of tumor microenvironment on the different breast cancer subtypes and applied therapies. *Biochem Pharmacol*. 2024;223:116178. doi:10.1016/j.bcp.2024.116178
8. Zarrilli G, Businello G, Dieci MV, et al. The tumor microenvironment of primitive and metastatic breast cancer: Implications for novel therapeutic strategies. *Int J Mol Sci*. 2020;21(21):8102. doi:10.3390/ijms21218102
9. Wei R, Liu S, Zhang S, Min L, Zhu S. Cellular and extracellular components in tumor microenvironment and their application in early diagnosis of cancers. *Anal Cell Pathol*. 2020;2020:6283796. doi:10.1155/2020/6283796
10. Terry S, Savagner P, Ortiz-Cuaran S, et al. New insights into the role of EMT in tumor immune escape. *Mol Oncol*. 2017;11(7):824-846. doi:10.1002/1878-0261.12093
11. Bray SJ, Kafatos FC. Developmental function of Elf-1: an essential transcription factor during embryogenesis in *Drosophila*. *Genes Dev*. 1991;5(9):1672-1683. doi:10.1101/gad.5.9.1672
12. Ting SB, Wilanowski T, Cerruti L, Zhao LL, Cunningham JM, Jane SM. The identification and characterization of human Sister-of-Mammalian Grainyhead (SOM) expands the grainyhead-like family of developmental transcription factors. *Biochem J*. 2003;370(Pt 3):953-962. doi:10.1042/BJ20021476
13. Wilanowski T, Tuckfield A, Cerruti L, et al. A highly conserved novel family of mammalian developmental transcription factors related to *Drosophila* grainyhead. *Mech Dev*. 2002;114(1-2):37-50. doi:10.1016/s0925-4773(02)00046-1
14. Kokoszynska K, Ostrowski J, Rychlewski L, Wyrwicz LS. The fold recognition of CP2 transcription factors gives new insights into the function and evolution of tumor

- p53. Cell Cycle. 2008;7(18):2907-2915. doi:10.4161/cc.7.18.6680
15. Rifat Y, Parekh V, Wilanowski T, et al. Regional neural tube closure defined by the Grainy head-like transcription factors. *Dev Biol.* 2010;345(2):237-245. doi:10.1016/j.ydbio.2010.07.017
  16. Ting SB, Wilanowski T, Auden A, et al. Inositol- and folate-resistant neural tube defects in mice lacking the epithelial-specific factor Grhl-3. *Nat Med.* 2003;9(12):1513-1519. doi:10.1038/nm961
  17. Kersbergen A, Best SA, Dworkin S, et al. Lung morphogenesis is orchestrated through Grainyhead-like 2 (Grhl2) transcriptional programs. *Dev Biol.* 2018;443(1):1-9. doi:10.1016/j.ydbio.2018.09.002
  18. Varma S, Cao Y, Tagne JB, et al. The transcription factors Grainyhead-like 2 and NK2-homeobox 1 form a regulatory loop that coordinates lung epithelial cell morphogenesis and differentiation. *J Biol Chem.* 2012;287(44):37282-37295. doi:10.1074/jbc.M112.408401
  19. Cangkrama M, Darido C, Georgy SR, et al. Two ancient gene families are critical for maintenance of the mammalian skin barrier in postnatal life. *J Invest Dermatol.* 2016;136(7):1438-1448. doi:10.1016/j.jid.2016.02.806
  20. Ting SB, Caddy J, Wilanowski T, et al. The epidermis of grhl3-null mice displays altered lipid processing and cellular hyperproliferation. *Organogenesis.* 2005;2(2):33-35. doi:10.4161/org.2.2.2167
  21. Senga K, Mostov KE, Mitaka T, Miyajima A, Tanimizu N. Grainyhead-like 2 regulates epithelial morphogenesis by establishing functional tight junctions through the organization of a molecular network among claudin3, claudin4, and Rab25. *Mol Biol Cell.* 2012;23(15):2845-2855. doi:10.1091/mbc.E12-02-0097
  22. Caddy J, Wilanowski T, Darido C, et al. Epidermal wound repair is regulated by the planar cell polarity signaling pathway. *Dev Cell.* 2010;19(1):138-147. doi:10.1016/j.devcel.2010.06.008
  23. Wang Z, Coban B, Liao CY, Chen YJ, Liu Q, Danen EHJ. GRHL2 regulation of growth/motility balance in luminal versus basal breast cancer. *Int J Mol Sci.* 2023;24(3):2512. doi:10.3390/ijms24032512
  24. Jolly MK, Tripathi SC, Jia D, et al. Stability of the hybrid epithelial/mesenchymal phenotype. *Oncotarget.* 2016;7(19):27067-27084. doi:10.18632/oncotarget.8166
  25. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest.* 2009;119(6):1420-1428. doi:10.1172/JCI39104
  26. Kim DH, Xing T, Yang Z, Dudek R, Lu Q, Chen YH. Epithelial mesenchymal transition in embryonic development, tissue repair and cancer: A comprehensive overview. *J Clin Med.* 2017;7(1). doi:10.3390/jcm7010001
  27. MacFawn I, Farris J, Pifer P, et al. Grainyhead-like-2, an epithelial master programmer, promotes interferon induction and suppresses breast cancer recurrence. *Mol Immunol.* 2024;170:156-169. doi:10.1016/j.molimm.2024.04.012

28. He Y, Gan M, Wang Y, et al. EGFR-ERK induced activation of GRHL1 promotes cell cycle progression by up-regulating cell cycle related genes in lung cancer. *Cell Death Dis.* 2021;12(5):430. doi:10.1038/s41419-021-03721-9
29. Kawabe N, Matsuoka K, Komeda K, et al. Silencing of GRHL2 induces epithelial-to-mesenchymal transition in lung cancer cell lines with different effects on proliferation and clonogenic growth. *Oncol Lett.* 2023;26(3):391. doi:10.3892/ol.2023.13977
30. Chung VY, Tan TZ, Ye J, et al. The role of GRHL2 and epigenetic remodeling in epithelial-mesenchymal plasticity in ovarian cancer cells. *Commun Biol.* 2019;2(1):272. doi:10.1038/s42003-019-0506-3
31. Hu F, He Z, Sun C, Rong D. Knockdown of GRHL2 inhibited proliferation and induced apoptosis of colorectal cancer by suppressing the PI3K/Akt pathway. *Gene.* 2019;700:96-104. doi:10.1016/j.gene.2019.03.051
32. Wang XK, Zhou FF, Tao HR, et al. Knockdown of GRHL3 inhibits activities and induces cell cycle arrest and apoptosis of human colorectal cancer cells. *J Huazhong Univ Sci Technol Med Sci.* 2017;37(6):880-885. doi:10.1007/s11596-017-1821-x
33. Mlacki M, Darido C, Jane SM, Wilanowski T. Loss of Grainy head-like 1 is associated with disruption of the epidermal barrier and squamous cell carcinoma of the skin. *PLoS One.* 2014;9(2):e89247. doi:10.1371/journal.pone.0089247
34. Kikulska A, Rausch T, Krzywinska E, et al. Coordinated expression and genetic polymorphisms in Grainyhead-like genes in human non-melanoma skin cancers. *BMC Cancer.* 2018;18(1):23. doi:10.1186/s12885-017-3943-8
35. Fabian J, Lodrini M, Oehme I, et al. GRHL1 acts as tumor suppressor in neuroblastoma and is negatively regulated by MYCN and HDAC3. *Cancer Res.* 2014;74(9):2604-2616. doi:10.1158/0008-5472.CAN-13-1904
36. Frisch SM, Farris JC, Pifer PM. Roles of Grainyhead-like transcription factors in cancer. *Oncogene.* 2017;36(44):6067-6073. doi:10.1038/onc.2017.178
37. Gasperoni JG, Fuller JN, Darido C, Wilanowski T, Dworkin S. Grainyhead-like (grhl) target genes in development and cancer. *Int J Mol Sci.* 2022;23(5):2735. doi:10.3390/ijms23052735
38. Kotarba G, Taracha-Wisniewska A, Wilanowski T. Grainyhead-like transcription factors in cancer - Focus on recent developments. *Exp Biol Med Maywood.* 2020;245(5):402-410. doi:10.1177/1535370220903009
39. Xu H, Liu C, Zhao Z, et al. Clinical implications of GRHL3 protein expression in breast cancer. *Tumour Biol.* 2014;35(3):1827-1831. doi:10.1007/s13277-013-1244-7
40. Zhao P, Guo S, Tu Z, et al. Grhl3 induces human epithelial tumor cell migration and invasion via downregulation of E-cadherin. *Acta Biochim Biophys Sin Shanghai.* 2016;48(3):266-274. doi:10.1093/abbs/gmw001
41. Tan L, Qu W, Wu D, et al. GRHL3 promotes tumor growth and metastasis via the MEK pathway in colorectal cancer. *Anal Cell Pathol.* 2021;2021:6004821. doi:10.1155/2021/6004821
42. Bhandari A, Gordon W, Dizon D, et al. The Grainyhead transcription factor Grhl3/Get1 suppresses miR-21 expression and tumorigenesis in skin: modulation

- of the miR-21 target MSH2 by RNA-binding protein DND1. *Oncogene*. 2013;32(12):1497-1507. doi:10.1038/onc.2012.168
43. Dompe N, Rivers CS, Li L, et al. A whole-genome RNAi screen identifies an 8q22 gene cluster that inhibits death receptor-mediated apoptosis. *Proc Natl Acad Sci U A*. 2011;108(43):E943-51. doi:10.1073/pnas.1100132108
44. Pan X, Zhang R, Xie C, et al. GRHL2 suppresses tumor metastasis via regulation of transcriptional activity of RhoG in non-small cell lung cancer. *Am J Transl Res*. 2017;9(9):4217-4226.
45. Nishino H, Takano S, Yoshitomi H, et al. Grainyhead-like 2 (GRHL2) regulates epithelial plasticity in pancreatic cancer progression. *Cancer Med*. 2017;6(11):2686-2696. doi:10.1002/cam4.1212
46. Cieply B, Riley P 4th, Pifer PM, et al. Suppression of the epithelial-mesenchymal transition by Grainyhead-like-2. *Cancer Res*. 2012;72(9):2440-2453. doi:10.1158/0008-5472.CAN-11-4038
47. Chen W, Yi JK, Shimane T, et al. Grainyhead-like 2 regulates epithelial plasticity and stemness in oral cancer cells. *Carcinogenesis*. 2016;37(5):500-510. doi:10.1093/carcin/bgw027
48. Xiang J, Fu X, Ran W, Wang Z. Grhl2 reduces invasion and migration through inhibition of TGF $\beta$ -induced EMT in gastric cancer. *Oncogenesis*. 2017;6(1):e284. doi:10.1038/oncsis.2016.83
49. Chen W, Kang KL, Alshaikh A, et al. Grainyhead-like 2 (GRHL2) knockout abolishes oral cancer development through reciprocal regulation of the MAP kinase and TGF- $\beta$  signaling pathways. *Oncogenesis*. 2018;7(5):38. doi:10.1038/s41389-018-0047-5
50. Xiang X, Deng Z, Zhuang X, et al. Grhl2 determines the epithelial phenotype of breast cancers and promotes tumor progression. *PLoS One*. 2012;7(12):e50781. doi:10.1371/journal.pone.0050781
51. Quan Y, Jin R, Huang A, et al. Downregulation of GRHL2 inhibits the proliferation of colorectal cancer cells by targeting ZEB1. *Cancer Biol Ther*. 2014;15(7):878-887. doi:10.4161/cbt.28877
52. Gregory PA, Bracken CP, Smith E, et al. An autocrine TGF-beta/ZEB/miR-200 signaling network regulates establishment and maintenance of epithelial-mesenchymal transition. *Mol Biol Cell*. 2011;22(10):1686-1698. doi:10.1091/mbc.E11-02-0103
53. Nie Y, Ding Y, Yang M. GRHL2 upregulation predicts a poor prognosis and promotes the resistance of serous ovarian cancer to cisplatin. *Onco Targets Ther*. 2020;13:6303-6314. doi:10.2147/OTT.S250412
54. Kang X, Chen W, Kim RH, Kang MK, Park NH. Regulation of the hTERT promoter activity by MSH2, the hnRNPs K and D, and GRHL2 in human oral squamous cell carcinoma cells. *Oncogene*. 2009;28(4):565-574. doi:10.1038/onc.2008.404
55. Tam WL, Weinberg RA. The epigenetics of epithelial-mesenchymal plasticity in cancer. *Nat Med*. 2013;19(11):1438-1449. doi:10.1038/nm.3336
56. Kotian S, Carnes RM, Stern JL. Enhancing transcriptional reprogramming of mesenchymal glioblastoma with Grainyhead-like 2 and HDAC inhibitors leads to



- apoptosis and cell-cycle dysregulation. *Genes Basel*. 2023;14(9). doi:10.3390/genes14091787
57. Pantelaiou-Prokaki G, Mieczkowska I, Schmidt GE, et al. HDAC8 suppresses the epithelial phenotype and promotes EMT in chemotherapy-treated basal-like breast cancer. *Clin Epigenetics*. 2022;14(1):7. doi:10.1186/s13148-022-01228-4
58. Werner S, Frey S, Riethdorf S, et al. Dual roles of the transcription factor grainyhead-like 2 (GRHL2) in breast cancer. *J Biol Chem*. 2013;288(32):22993-23008. doi:10.1074/jbc.M113.456293
59. Yang X, Vasudevan P, Parekh V, Penev A, Cunningham JM. Bridging cancer biology with the clinic: relative expression of a GRHL2-mediated gene-set pair predicts breast cancer metastasis. *PLoS One*. 2013;8(2):e56195. doi:10.1371/journal.pone.0056195
60. Holding AN, Giorgi FM, Donnelly A, et al. Correction to: VULCAN integrates ChIP-seq with patient-derived co-expression networks to identify GRHL2 as a key co-regulator of ERα at enhancers in breast cancer. *Genome Biol*. 2019;20(1):122. doi:10.1186/s13059-019-1733-0
61. Cocce KJ, Jasper JS, Desautels TK, et al. The lineage determining factor GRHL2 collaborates with FOXA1 to establish a targetable pathway in endocrine therapy-resistant breast cancer. *Cell Rep*. 2019;29(4):889-903.e10. doi:10.1016/j.celrep.2019.09.032
62. Reese RM, Helzer KT, Allen KO, Zheng C, Solodin N, Alarid ET. GRHL2 enhances phosphorylated estrogen receptor (ER) chromatin binding and regulates ER-mediated transcriptional activation and repression. *Mol Cell Biol*. 2022;42(10):e0019122. doi:10.1128/mcb.00191-22
63. Wellenstein MD, de Visser KE. Cancer-cell-intrinsic mechanisms shaping the tumor immune landscape. *Immunity*. 2018;48(3):399-416. doi:10.1016/j.immuni.2018.03.004
64. Salemme V, Centonze G, Cavallo F, Defilippi P, Conti L. The crosstalk between tumor cells and the immune microenvironment in breast cancer: Implications for immunotherapy. *Front Oncol*. 2021;11:610303. doi:10.3389/fonc.2021.610303
65. Binnewies M, Roberts EW, Kersten K, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat Med*. 2018;24(5):541-550. doi:10.1038/s41591-018-0014-x
66. Dongre A, Rashidian M, Reinhardt F, et al. Epithelial-to-mesenchymal transition contributes to immunosuppression in breast carcinomas. *Cancer Res*. 2017;77(15):3982-3989. doi:10.1158/0008-5472.CAN-16-3292
67. Hugo W, Zaretsky JM, Sun L, et al. Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. *Cell*. 2016;165(1):35-44. doi:10.1016/j.cell.2016.02.065
68. Khadri FZ, Issac MSM, Gaboury LA. Impact of epithelial-mesenchymal transition on the immune landscape in breast cancer. *Cancers Basel*. 2021;13(20):5099. doi:10.3390/cancers13205099

69. Song S, Zhang D, Chen J, et al. CHMP4A stimulates CD8<sup>+</sup> T-lymphocyte infiltration and inhibits breast tumor growth via the LSD1/IFN $\beta$  axis. *Cancer Sci.* 2023;114(8):3162-3175. doi:10.1111/cas.15844
70. MacFawn I, Wilson H, Selth LA, et al. Grainyhead-like-2 confers NK-sensitivity through interactions with epigenetic modifiers. *Mol Immunol.* 2019;105:137-149. doi:10.1016/j.molimm.2018.11.006
71. Liang Y, Liu Y, Zhang Q, Zhang H, Du J. Corrigendum to Tumor-derived extracellular vesicles containing microRNA-1290 promote immune escape of cancer cells through the Grhl2/ZEB1/PD-L1 axis in gastric cancer [Translational Research 231C (2021)102-112]. *Transl Res.* 2022;247:168. doi:10.1016/j.trsl.2021.12.010