Minireview

Recent discoveries concerning the involvement of transcription factors from the Grainyhead-like family in cancer

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Abstract

The Grainyhead-like (GRHL) family of transcription factors has three mammalian members, which are currently termed Grainyhead-like 1 (GRHL1), Grainyhead-like 2 (GRHL2), and Grainyhead-like 3 (GRHL3). These factors adopt a DNA-binding immunoglobulin fold homologous to the DNA-binding domain of key tumor suppressor p53. Their patterns of expression are tissue and developmentally specific. Earlier studies of the GRHL proteins focused on their functions in mammalian development. In recent years, these factors have been linked to many different types of cancer: squamous cell carcinoma of the skin, breast cancer, gastric cancer, hepatocellular carcinoma, colorectal cancer, clear cell renal cell carcinoma, neuroblastoma, prostate cancer, and cervical cancer. The roles of GRHL proteins in these various types of cancer are complex, and in some cases appear to be contradictory: they can serve to promote cancer development, or they may act as tumor suppressors, depending on the particular GRHL protein involved and on the cancer type. The reasons for obvious discrepancies in results from different studies remain unclear. At the molecular level, the GRHL transcription factors regulate the expression of genes whose products are involved in cellular proliferation, differentiation, adhesion, and polarity. We herein review the roles of GRHL proteins in cancer development, and we critically examine relevant molecular mechanisms, which were proposed by different authors. We also discuss the significance of recent discoveries implicating the involvement of GRHL transcription factors in cancer and highlight potential future applications of this knowledge in cancer treatment.

Keywords: Cancer, epithelium, Grainyhead-like factors, transcription factors, gene expression, oncogenes, tumor suppressors

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Introduction

The Grainyhead-like (GRHL) proteins constitute a family of transcription factors whose first member, Grainyhead (GRH), was discovered in the fruit fly *Drosophila melanogaster*.[1–3] These factors adopt a DNA-binding immunoglobulin fold homologous to the core domain of key tumor suppressor p53. With a traceable evolution of GRHL throughout the Metazoa group, this protein family is likely to represent an ancestor of p53.[4]

In mammals there are three members of the GRHL family, which are currently termed GRHL1–3.[5,6] The expression of these factors is tissue and developmentally specific. They are found primarily in epithelial tissues, in organs such as epidermis, oral and olfactory epithelium, kidneys and urogenital tract, stomach and the digestive tract, heart and lung.[7] Numerous mouse models have been engineered in order to investigate the roles of GRHL transcription factors in mammals. These included knockout and conditional knockout strains as well as mutants generated using other methods. The analysis of these models provided fundamental information about the functions of GRHL proteins in mammals. The GRHL1 transcription factor is important for the functioning of the epidermis, as the *Grhl1*$^{-/-}$ mice exhibit palmoplantar keratoderma, impaired hair anchoring, and desmosomal abnormalities.[8] Loss of *Grhl2* causes early embryonic lethality with severe neural tube closure defects.[9–11] The GRHL3 factor is essential for neural tube closure, epidermal barrier formation, and wound healing, and the *Grhl3*-null mice die at birth with spina bifida and severe skin barrier defects.[12–14]

Involvement of GRHL factors in cancer

Grainyhead-like 1 (GHRL1)

GRHL1 is a tumor suppressor in the squamous cell carcinoma (SCC) of the skin. When subjected to a standard chemical skin carcinogenesis protocol, *Grhl1*$^{-/-}$ mice develop significantly more SCC tumors than their *Grhl1*$^{+/+}$ control

littermates. The underlying mechanism is associated with aberrant terminal differentiation of keratinocytes and mild chronic skin inflammation in the *Grhl1*-null mice.[15]

Neuroblastoma is an early childhood neuroendocrine tumor originating from neural crest cells. Data obtained from primary tumors show that high levels of *GRHL1* expression correlate with favorable prognosis for patients. When *GRHL1* is ectopically expressed in those neuroblastoma cell lines that have low endogenous *GRHL1* levels, this abolishes anchorage-independent colony formation, inhibits cell proliferation, and retards xenograft growth in mice. On the other hand, when the expression of *GRHL1* is knocked down by RNA interference in those neuroblastoma cell lines that have high endogenous *GRHL1* levels, it increased their capacity for growth in soft agar. Taken together, all these lines of evidence support the notion that the GRHL1 transcription factor acts as a tumor suppressor in neuroblastoma.[16]

Grainyhead-like 2 (GRHL2)

Research carried out by several scientific teams provided evidence for the involvement of GRHL2 transcription factor in breast cancer. GRHL2 is downregulated specifically in the claudin-low subclass breast tumors and in basal-B subclass breast cancer cell lines, where it suppresses epithelial–mesenchymal transitions (EMT), enhances anoikis sensitivity, and suppresses mammosphere generation in mammary epithelial cells.[17] GRHL2 expression prevents tumor initiation in xenograft assays, sensitizes breast cancer cells to paclitaxel, and suppresses the emergence of CD44(high)CD24(low) cells (defining the cancer stem cell phenotype in the cell type studied). GRHL2 is downregulated in recurrent mouse tumors that had evolved to an oncogene-independent, EMT-like state, supporting a role for GRHL2 downregulation in this phenotypic transition, modeling disease recurrence.[18] These effects are mediated in part by suppression of zinc finger E-box binding homeobox 1 (ZEB1) expression via direct repression of the *ZEB1* promoter. GRHL2 also inhibits Smad-mediated transcription and upregulates miR-200b/c as well as the transforming growth factor beta (TGFβ) receptor antagonist, bone morphogenetic protein 2 (BMP2). Ectopic expression of *GRHL2* in MDA-MB-231 breast cancer cell line triggers reversion of EMT and restores sensitivity to anoikis.[17] The combination of TGFβ and WNT activation represses *GRHL2* expression by direct interaction of ZEB1 with the *GRHL2* promoter, inducing EMT. Taken together, these observations indicate that a reciprocal feedback loop between GRHL2 and ZEB1 controls epithelial versus mesenchymal phenotypes and EMT-driven tumor progression.[18]

GRHL2 directly regulates the expression of E-cadherin, which is a key suppressor of invasion and metastasis in cancer and its downregulation has been linked to the progression of neoplasms.[9,11] Knockdown of *GRHL2* expression by shRNA in human mammary epithelial cell line MCF10A leads to downregulation of E-cadherin and EMT.[19] Other putative GRHL2 target genes that may be relevant to breast cancer include N-cadherin, alphaT-catenin, beta-catenin, fibronectin 1, CITED2, and ERBB3.[20,21] In contradiction to these data, over-expression of Grhl2 in murine mammary gland tumor cell line 4T1 induces epithelial gene expression and promotes tumor growth and metastasis in a mouse model.[19] Furthermore, increased expression of GRHL2 correlates with poor relapse-free survival and increased risk of metastasis in breast cancer patients.[19,21] At present, the reasons for obvious discrepancies in results from different studies are unclear.[20] However, a similar paradox was observed in the case of TGFβ, which can function as a tumor suppressor in normal and early stage cancers and has tumor promoter activity in the late stage cancers. Given the parallels between mesenchymal-to-epithelial transition (MET) programs induced by BMPs and GRHL2, future studies need to determine the extent to which GRHL2 mediates MET reactions stimulated by BMPs, and conversely, how these events are inactivated by TGFβ in developing and progressing carcinoma cells.[22]

The reciprocal feedback loop between GRHL2 and ZEB1, described above for breast cancer, has also been found in colorectal cancer.[23,24] Expression of GRHL2 is increased in colorectal cancer samples and is correlated with higher levels of Ki-67 staining, larger tumor size, and advanced clinical stage. Knocking down of *GRHL2* expression in HT29 and HCT116 colorectal cancer cell lines significantly inhibited cell proliferation *in vitro* and tumorigenesis *in vivo*. The underlying molecular mechanism involves the regulation of expression of ZEB1 and E-cadherin,[23] akin to the mechanism proposed for GRHL2 involvement in breast cancer.[18]

There are conflicting reports regarding the role of *GRHL2* in gastric cancer. The *GRHL2* gene is located in the chromosomal region 8q22.3. This region is frequently amplified in many cancers, including gastric cancer. As a consequence, *GRHL2* is often over-expressed in gastric cancer samples and under-expressed in matched adjacent non-cancerous samples.[25] However, a different research team published contradictory results. Both mRNA and protein expression levels of GRHL2 were found to be significantly downregulated in gastric cancer samples. Exogenous *GRHL2* transduced into human gastric cancer cell line SGC7901 significantly inhibited proliferation and promoted apoptosis. Meanwhile, over-expression of *GRHL2* decreased c-Myc and Bcl-2 protein expression levels. The authors concluded that *GRHL2* may function as a tumor suppressor and play an important role in the development and progression of gastric cancer.[26] Once again, the reasons for discrepancies in results from different research groups are unclear.

GRHL2 was found to interact with the promoter of human telomerase reverse transcriptase (*hTERT*). Higher expression of *hTERT* and subsequent activation of telomerase occur during cellular immortalization and are maintained in cancer cells. The expression of *GRHL2* is higher in human oral SCC cells than in normal cells, which do not exhibit telomerase activity. Knockdown of *GRHL2* results in a notable reduction of *hTERT* promoter activity in tested cancer cells. Silencing of *GRHL2* is essential in reducing telomerase activity and viability of SCC cells. These results suggest a possible role for *GRHL2* in telomerase activation during cellular immortalization.[27]

Of other cancer types, a gain of *GRHL2* is associated with early recurrence of hepatocellular carcinoma progression, while decreased *GRHL2* expression by RNA interference inhibits the growth of hepatoma cells, suggesting its association with cell proliferation. A gain of *GRHL2* might thus be a predictive marker for hepatocellular carcinoma recurrence.[28] In clear cell renal cell carcinoma, *GRHL2* expression is associated with higher risk of disease relapse and *GRHL2*-positive patients have significantly lower disease-free survival.[29] A loss or strong reduction in *GRHL2* was observed in cervical cancer samples, suggesting that downregulation of *GRHL2* is a necessary step during cervical carcinogenesis.[30] Increased expression of *GRHL2* has been found in metastatic prostate cancer samples.[31] However, molecular mechanisms responsible for the involvement of *GRHL2* in these types of cancer remain to be elucidated.

Grainyhead-like 3 (GRHL3)

GRHL3 serves a protective role against SCC of the skin both in an animal model and in human patients.[32,33] Mice, in which the expression of *Grhl3* is selectively abolished only in the adult epidermis, exhibit increased susceptibility to chemically induced skin carcinogenesis, as well as spontaneous skin tumor formation. Tumor suppressor phosphatase and tensin homolog (PTEN) is a direct target of GRHL3 regulation in the skin, and consequently reduced expression of *Pten* is observed in the *Grhl3*-deficient epidermis. This decrease in PTEN levels leads to the activation of PI3K/AKT/mTOR signaling, which is the cause of increased SCC occurrence in the *Grhl3* conditional knockout mice. Furthermore, the expression of *GRHL3* and *PTEN* is reduced in human SCC skin samples. The proto-oncogenic microRNA miR-21 synchronously targets both *GRHL3* and *PTEN* in human skin tumors and is responsible for the decrease in their levels of expression.[33] In addition, GRHL3 binds to the miR-21 promoter and represses its expression, so these two factors are involved in a regulatory loop maintaining homeostasis in the epidermis.[32]

GRHL3 has also been implicated in breast cancer. In an early report, it was suggested that zinc finger protein 652 represses *GRHL3* in normal breast epithelial cells but this repression is lost or inhibited in cancer.[34] This would imply a pro-tumorigenic role for this transcription factor in breast cancer. However, later research failed to support this hypothesis. GRHL3 is highly expressed in the early stages of breast cancer, which was observed both in tumor and plasma samples, but its expression is greatly reduced in advanced stages of this disease.[35] In another study, GRHL3-positive breast cancer cases correlated with a favorable prognosis, while GRHL3-negative cases had a poor prognosis. In cases with lymph node invasion, GRHL3 also contributed to longer survival times. All these results strongly suggest an inhibitory effect of GRHL3 in breast cancers and the possible involvement of GRHL3 in tumor suppression.[36] The underlying mechanism may involve direct regulation of E-cadherin by GRHL3. GRHL3 positively regulates the expression of E-cadherin during MET in non-tumorigenic mouse mammary gland cells as well as in many cancer cell lines, breast cancer specimens, and in lung cancer.[37]

GRHL3 is abundantly expressed in advanced stages of two subtypes of lung cancer: small cell lung cancer and adenocarcinoma.[38] Its role in these types of cancer is currently unclear. In contradiction to these findings, in a recent publication it was reported that GRHL3 positively regulates the expression of E-cadherin in lung cancer, which would be suggestive of a tumor suppressive function.[37] However, the authors of the latter report did not specify the subtype of lung cancer studied, so it is still possible that GRHL3 acts as a tumor suppressor in some types of lung cancer and fulfils a different function in other types of lung cancer.

Role of GRHL factors in epithelial tissues

The expression of all the GRHL1-3 transcription factors is tissue and developmentally specific and occurs predominantly in the epithelial tissues.[7] The role of GRHL proteins in maintaining epithelial integrity is conserved throughout Metazoa; it is noteworthy that the evolutionary origin of GRHL factors appears roughly coincident with the evolutionary origin of the epithelium.[39]

In a recent analysis of molecular interaction networks involved in the proper functioning of the epithelia, the GRHL1 and GRHL2 transcription factors emerged with the largest number of interactions impacting cell surface complexes, suggesting key roles in the functioning of epithelial cell–cell junctions.[40] Experiments using mouse models confirmed the importance of GRHL proteins in epithelial tissues. The GRHL1 transcription factor is necessary for the proper functioning of the epidermis, as the *Grhl1*-null mice exhibit symptoms reminiscent of palmoplantar keratoderma, severely deregulated terminal differentiation of keratinocytes, as well as mild chronic skin barrier defects.[8,15]

An example of process involving epithelia is neural tube closure, and both *Grhl2* and *Grhl3* are indispensable for neural tube closure in a mouse model.[9–11,13] *Grhl3* is also essential for eyelid closure[41,42] and wound healing.[12,43] Other epithelial processes involving *Grhl3* include formation of an impermeable barrier in the epidermis[12,14] and in the urinary bladder.[44]

In the liver, GRHL2 regulates epithelial morphogenesis, enhances epithelial barrier of duct tubules, and controls lumen expansion by regulating the expression of proteins involved in tight junctions.[45] GRHL2 is also necessary for barrier formation and control of lumen expansion in renal epithelia.[46] In human patients, mutations in the *GRHL2* gene cause an autosomal-recessive ectodermal dysplasia syndrome.[47] In addition, GRHL2 is essential for the functioning of lung epithelia: it is crucial for the morphogenesis of lung epithelial cells and it regulates many physiological functions of human airway epithelium.[48,49] Furthermore, GRHL2 controls trophoblast branching morphogenesis in the placenta.[50] Taken together, all the above data indicate essential functions of the GRHL transcription factors in the development and maintenance of epithelia.

Known functions of GRHL transcription factors in cancer and in epithelia are summarized in Table 1.

Table 1 Summary of literature links between GRHL transcription factors, their roles in epithelia and in various types of cancer; question marks indicate links as yet undiscovered, but predicted by us

Transcription factor	Epithelium	Cancer
Grainyhead-like 1 (GRHL1)	Epidermis[8,15]	Skin cancer[15]
	?	Neuroblastoma[16]
Grainyhead-like 2 (GRHL2)	Mammary gland ducts[17,19,20]	Breast cancer[17–21]
	Colon and rectum[7]	Colorectal cancer[23,24]
	Lining of the stomach[26]	Gastric cancer[25,26]
	Oral epithelium[7]	Oral squamous cell carcinoma[27]
	Liver ducts[45]	Hepatocellular carcinoma[28]
	Renal epithelium[46]	Clear cell renal cell carcinoma[29]
	Cervix[30]	Cervical cancer[30]
	?	Prostate cancer[31]
	Epidermis[47]	?
	Lung airways[48,49]	?
	Placenta[50]	?
Grainyhead-like 3 (GRHL3)	Epidermis[12,14]	Skin cancer[32,33]
	Mammary gland ducts[37]	Breast cancer[34–36]
	Lung airways[7]	Lung cancer[37,38]
	Urinary bladder[44]	?

Conclusions

Almost all the cancers mentioned in this manuscript are of epithelial origin. EMTs are known to play a critical role during tumor metastasis, which generates cells with migratory and invasive properties that are able to disseminate to distant organs.[51,52] However, the molecular mechanisms of EMT during tumor progression are not yet fully understood. On the basis of known functions of the GRHL transcription factors, we propose a general mechanistic principle governing the involvement of these factors in cancer. The GRHL proteins are essential for the development and maintenance of various epithelia. They are involved in the regulation of relevant target genes and signaling pathways. Consequently, disruptions in the expression of GRHL proteins interfere with proper functioning of the epithelia, support EMT and other processes that favor tumor development and progression.

Future perspectives

If the above hypothesis is true, then we can predict discovery of hitherto unknown links between the GRHL transcription factors and cancers of epithelial origin, derived from epithelia in which these factors are expressed.[7] Such cancers may include: esthesioneuroepithelioma, also known as olfactory neuroblastoma (a tumor of olfactory epithelium origin); esophageal cancer; salivary gland cancer; choriocarcinoma (a cancer of the placenta); urinary bladder cancer; and others.

In the future, information about the status of *GRHL* genes may prove useful in prevention and treatment of various cancers. From the studies of mouse models we know that reduced expression of *Grhl1* and *Grhl3* genes increases the risk of skin cancer.[15,33] Accordingly, people harboring mutations that alter the expression of *GRHL* genes or modify the functioning of GRHL proteins may have an increased risk of developing cancer. Also, silencing of *GRHL* genes in non-tumorigenic cell lines induces tumorigenic features in these cells, such as enhanced proliferation, reduced apoptosis, and increased colony growth in soft agar. Conversely, increasing the expression of these genes in cancer cell lines reverses their tumorigenic phenotype. This has already been proven true for *GRHL1* in neuroblastoma,[16] *GRHL2* in gastric cancer,[26] and *GRHL3* in skin cancer.[33] However, in the case of *GRHL2* in colorectal cancer, an opposite situation was observed: knocking down of *GRHL2* in colorectal cancer cells inhibited their proliferation and tumorigenesis in a mouse xenograft model.[23] For these reasons, targeted therapies aimed at altering *GRHL* gene expression or GRHL protein function in cancers may prove beneficial in cancer treatment in the future.

Very little is currently known about interactions between different GRHL transcription factors, but such interactions have already been discovered. For example, GRHL2 inhibits the expression of GRHL1 and GRHL3 in normal human epidermal keratinocytes.[53] Such mutual interactions may be crucial for our understanding of the functions of these factors in various processes including cancer, therefore research into this topic is urgently needed.

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