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Review

GRIM-19 function in cancer development

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ABSTRACT

Cancer development involves multiple genetic changes, which can occur in tumor suppressor genes and lead to loss of function in a recessive manner. Recent findings have identified a novel tumor suppressor gene named GRIM-19. Similar to what has been observed for other known tumor suppressor proteins such as p53, GRIM-19 gene mutations and loss of protein expression have been observed in several tumor types. In this review, we perform a detailed description on the current understanding of GRIM-19 function in carcinogenesis.

Contents

1. Introduction

Gene associated with retinoid-IFN-induced mortality (GRIM)-19 was identified by a research group interested in unveiling the molecular basis of cell death associated with exposure to interferon (IFN)-β and retinoic acid (RA) (Angell et al., 2000). IFNs and RA have both been shown to suppress tumor growth and are used in the treatment of several cancers (Altucci and Gronemeyer, 2001; Gresser and Belardelli, 2002; Ikeda et al., 2002). By employing an anti-sense knock-out approach, the group was able to identify several genes associated with IFN-β and RA-induced mortality, including GRIM-19 (Angell et al., 2000). In breast carcinoma cell lines, both GRIM-19 mRNA and protein expression increased in the presence of IFN-β/RA. Further experiments

Abbreviations: GRIM-19, Gene associated with retinoid-IFN-induced mortality; IFN, interferon; RA, retinoic acid; MRC, mitochondrial respiratory chain; OXPHOS, oxidative phosphorylation; TCO, tumors with cell oxyphilia; RCC, renal cell carcinoma; STAT3, signal transducer and activator of transcription 3; sh, short hairpin; Bcl, B-cell lymphoma protein, mcl-1, myeloid cell leukemia sequence 1; TAD, transactivation domain; S, serine; T, tyrosine; V, valine; Q, glutamic acid; D, aspartate; M, methionine; P, proline; K, lysine; N, asparagine; OLFM4, Olfactomedin 4; NF-κB, nuclear factor-κB; MMP, matrix metalloproteinase; u-PA, urokinase-type plasminogen activator; VEGF, vascular endothelial growth factor; CDK, cyclin-dependent kinase; INK, Inhibitor of CDK kinase; RB, retinoblastoma; SDH, succinate dehydrogenase; PGL, paragangliomas; HHV-8, human Herpesvirus-8; HPV, human Papillomavirus; HHV-6B, human Herpesvirus-6B; CMV, Cytomegalovirus; VV, Vaccinia virus; HIV-1, human Immunodeficiency virus-1; NOD2, Nucleotide-binding Oligomerization Domain containing 2.

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to characterize GRIM-19 protein showed that its overexpression induced apoptotic cell death in response to IFN-β/RA treatment, while GRIM-19 knock-down seemed to confer cell resistance to these factors. In this context, GRIM-19 inhibition confers some growth advantage. By immunofluorescence, GRIM-19 was shown to localize predominantly in the nucleus of transfected HeLa cells, although some punctate cytoplasmic staining could also be observed. In addition, a study by Chidambaram et al. (2000) described the localization of GRIM-19 to chromosome 19p13.2, a region crucial for prostate tumor suppression and with loss of heterozygosity in sporadic and familial forms of thyroid cancer (Prazeres et al., 2008; Stankov et al., 2004). Altogether, these results led the authors to propose that GRIM-19 could be a novel tumor suppressor.

Studies performed by different groups demonstrated that GRIM-19 is also a component of complex I of the mitochondrial respiratory chain (MRC), which is responsible for ATP production (Fearnley et al., 2001; Hu et al., 2002; Murray et al., 2003). Indeed, GRIM-19 is essential for early embryonic development in mice as demonstrated by genetic ablation experiments. Homologous deletion of the GRIM-19 gene resulted in embryonic lethality by day 9.5, partially due to oxidative phosphorylation (OXPHOS) failure (Huang et al., 2004). In addition, GRIM-19 knock-out blastocysts not only showed retarded growth *in vitro* but also exhibited abnormal mitochondrial structure, morphology and distribution. Careful analysis of GRIM-19 localization in several cell types indicated that this protein is mainly present in the mitochondria and that its elimination results in complex I disassembly and disruption of electron transfer (Huang et al., 2004). It is possible that this somewhat contradictory evidence regarding GRIM-19 localization within the cell may actually reflect different roles of this protein in cell biology (Maximo et al., 2008). It is now clear that GRIM-19 is a dual function gene that is involved in mitochondrial metabolism and IFN-β/RA-induced cell death. The involvement of mitochondria in cell death pathways may be the link between these functions (Maximo et al., 2008).

Although it is beyond the scope of the present review, it is important to stress that GRIM-19 appears also to participate in viral infection and innate immune response as its activity is modulated by several viruses and bacteria [for a thorough review see (Kalvakolanu et al., 2010)]. Briefly: the viral IFN regulatory factor 1 (vIRF1) from human Herpesvirus-8 (HHV-8) binds to GRIM-19 and blocks its ability to induce apoptosis (Seo et al., 2002); the human Papillomavirus (HPV) E6 protein of the high-risk strains, but not the low-risk strains, binds to GRIM-19 (Seo et al., 2002); the human Herpesvirus-6B (HHV-6B) U95 interacts with GRIM-19 (Yeo et al., 2008); the non-coding 2.7-kb viral RNA (β2.7) from Cytomegalovirus (CMV) interacts with GRIM-19 and regulates mitochondria induced cell death (Reeves et al., 2007); other viruses, such as Vaccinia virus (VV) and human Immunodeficiency virus-1 (HIV-1), appear to target GRIM-19 *via* different mechanisms (Guerra et al., 2003; Tripathy et al., 2010). These data suggest that GRIM-19 is a general target of viral proteins.

Furthermore, intra-cellular bacteria, such as *Salmonella typhimurium* and *Porphyromonas gingivalis*, appear also to regulate GRIM-19 expression (Barnich et al., 2005; Zhou and Amar, 2006). GRIM-19 has been shown to interact also with Nucleotide-binding Oligomerization Domain containing 2 (NOD2), suggesting that GRIM-19 may be a key component in NOD2-mediated innate mucosal response and serve to regulate cell responses to microbes (Barnich et al., 2005).

2. GRIM-19 gene mutations and loss of expression in tumors

The initial discovery of GRIM-19 as a growth suppressor in a genetic screen hinted that it could be a target for genetic inactivation events. This hint was supported by the finding of mutations in the coding region of the GRIM-19 gene in Hürthle cell thyroid carcinomas by our group (Maximo et al., 2005). Hürthle cell tumors' main feature is that they are formed by cells packed with abnormal mitochondria. Somatic missense mutations in the GRIM-19 gene were found in 3 out of 20 sporadic Hürthle cell carcinomas. In addition, 1 germline mutation in a Hürthle cell papillary carcinoma was identified in a thyroid with multiple Hürthle cell nodules. However, no mutations were detected in the 20 non-Hürthle cell tumors nor in the 96 blood samples analyzed (Table 1), strongly suggesting the involvement of GRIM-19 alterations in the etiopathogenesis of mitochondrion-rich thyroid tumors. Indeed, a previous report suggested that mutations in genes involved in mitochondrial function and consequently in cellular energy production could be responsible for the increase in mitochondrial number observed in Hürthle cell tumors (Katoh et al., 1998). This was proposed after the observation that a defect in the cells' energetic capacity can lead to a secondary increase in the number of mitochondria through a feedback mechanism (Attardi et al., 1995). Since GRIM-19 is an essential component of the MRC, it is possible that mutations in or deregulation of GRIM-19 protein is the reason for accumulation of mitochondria in these tumor cells, as a compensatory mechanism (Maximo et al., 2005). As the gene predisposing patients to thyroid tumors with cell oxyphilia (TCO) has been mapped to chromosome 19p13.2 (Canzian et al., 1998), the exact same location as that of the GRIM-19 gene, we hypothesized that GRIM-19 could be the TCO gene whose identity was unknown. However, the results obtained did not corroborate our hypothesis. Still, the mutations we have reported in the GRIM-19 gene remain the only nuclear gene mutations specific to Hürthle cell tumors identified to date.

In a study by Alchanati et al. (2006), the authors reported that GRIM-19 protein expression was lost or severely repressed in a number of primary renal cell carcinomas (RCC). Western blot analysis revealed that of the 11 clear cell RCCs analyzed, GRIM-19 protein expression was completely lost in 4, very weak in 6 and moderately decreased in 1 when compared with paired-normal tissue (Table 1). This finding was further supported by immunohistochemical experiments, which showed that GRIM-19 expression was completely absent in 27 of the 29 paraffin embedded RCC samples analyzed (20 of the clear cell type, 5 of the chromophobe type and 4 of the papillary type). The other 2 tumors exhibited weak positive staining (1 of the chromophobe type and the other of the clear cell type) when compared to the normal kidney tissue. Curiously, these results suggest that at least in RCCs, GRIM-19 alterations do not correlate with mitochondrion-enriched tumor cells. Studies performed by our group corroborate these observations (Fig. 1, unpublished results). GRIM-19 protein downregulation was linked with severe to complete loss of mRNA expression in tumors. Although very preliminary, the mRNA expression data together with the observation that GRIM-19 gene harbors no mutations in all cases analyzed but one (which had a conservative substitution) led the authors to suggest that GRIM-19 downregulation occurs at the transcriptional level (Alchanati et al., 2006). Analysis of GRIM-19 ablation in a RCC cell line revealed that cells electroporated with antisense GRIM-19 mRNA exhibited increased growth rates both *in vitro* and *in vivo*; this effect was at least in part, mediated by signal transducer and activator of transcription 3 (STAT3), as expression of STAT3-dependent genes was significantly increased (Alchanati et al., 2006).

More recently, various studies described loss or decrease in GRIM-19 protein expression in colorectal, prostate and cervical carcinomas. Gong et al. (2007) described that in 23 colorectal specimens analyzed, mRNA expression of GRIM-19 was clearly lower than in normal tissues. These observations were further corroborated by western blot and immunohistochemistry experiments, which also revealed an upregulation of STAT3 specifically in the carcinoma tissues (Table 1). Constitutive activation or overexpression of STAT3 has been detected in a variety of human tumors (Bromberg et al., 1999; Buettner et al., 2002, see later for further details). Zhang et al. (2008) demonstrated that GRIM-19 expression is also reduced in primary prostate cancer. By immunostaining, they found that GRIM-19 expression levels in primary prostate tumors were significantly lower than in normal prostate tissues. Interestingly, low GRIM-19 expression correlated