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Isolation and characterization of novel pituitary tumor related genes: A cDNA representational difference approach

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Abstract

Recently, progress has been made in understanding human pituitary tumor pathogenesis by the investigation of differences in gene expression between normal pituitary tissue and pituitary tumors. A number of approaches, including differential display (DD), representational difference analysis (RDA), and microarray analysis have been used and several molecular targets potentially associated with pituitary tumor development and invasion have been identified. We have used RDA to compare gene expression patterns between normal human pituitary and clinically non-functioning pituitary adenomas, and identified genes with growth suppression function which are expressed in the normal pituitary but not in pituitary tumors. In particular, we have focused on an imprinted gene, *Maternally Expressed Gene 3 (MEG3)*, which is specifically associated with clinically non-functioning pituitary adenomas of a gonadotroph lineage. *MEG3* functions to suppress tumor cell growth, increase protein expression of the tumor suppressor p53, and selectively activate p53 target genes. Interestingly, *MEG3* does not encode a protein but a non-coding RNA. Therefore, these studies have revealed novel mechanisms for the function of a non-coding RNA in pituitary physiology and tumorigenesis.

Keywords

cDNA representational difference analysis; clinically non-functioning pituitary tumors; maternally expressed gene 3 (MEG3)

Introduction

Pituitary tumors are monoclonal in origin and a somatic mutation is a requisite event in tumor formation [1,2]. The mechanisms underlying selective clonal proliferation in pituitary tumors and the identification of specific causal genetic rearrangements, deletions or mutations has been elusive in the vast majority of tumors. Common mutations in aggressive or malignant human tumors, such as p53 and Rb, are extremely rare in pituitary adenomas [3,4]. Other oncogenes and tumor suppressors, such as H-ras, MEN-1, and c-myc, have been investigated [5-11], and, with the exception of the hereditary MEN-1 syndrome, none of these mutations themselves are sufficient to explain pituitary tumor pathogenesis. One mutation consistently found in pituitary adenomas is a growth promoting oncogene, *gsp*, in a

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significant minority of somatotroph tumors [12]. Recently, mutations of the *AIP* gene (*aryl hydrocarbon receptor-interacting protein*) were found to be germline events predisposing patients to somatotroph adenomas [13-15]. However, *AIP* mutations were not found in sporadic pituitary tumors [16-18]. Therefore, genetic events underlying neoplastic transformation and proliferation remain to be identified for the majority of pituitary adenoma phenotypes. In addition to genetic mutations, epigenetic modifications, such as DNA methylation, have been shown to play an important role in gene silencing. For example, expression of tumor suppressors Rb and p16^{INK4a} were found to be reduced or completely silenced in many clinically non-functioning pituitary adenomas due to their promoter hypermethylation [19-22], indicating that the function of a gene can be affected by altering its expression level.

Up to 40% of pituitary tumors are not associated with hormone hypersecretion and have been classified as clinically non-functioning. These tumors can cause considerable morbidity because of mass effect [23,24] and unlike most secretory tumors, there are no established medical therapies to control tumor growth [25,26]. Although patients with non-functioning tumors do not have clinical syndromes related to hormone excess, the majority of such tumors synthesize one or more gonadotropins and/or their free α - and β -subunits and are considered to be of gonadotroph cell origin [27,28]. However, intact gonadotropin and free hormone subunit secretion is minimal compared to tumor mass and suppression of hormone secretion does not control tumor mass. The mechanisms controlling tumor growth in gonadotroph-derived tumors are not well understood. An understanding of pituitary tumor pathogenesis and specific factors regulating pituitary cell proliferation is key to the development of new therapeutic strategies for such tumors.

To identify novel factors involved in pituitary tumor pathogenesis, several studies have been focused on differences in gene expression between pituitary tumors and normal pituitary tissue. This review will focus on the identification and characterization of novel pituitary tumor related genes using representational difference analysis (RDA) and highlight one such gene, *Maternally Expressed Gene 3 (MEG3)*.

Investigation of differences in gene expression between normal pituitary and tumors

Before the development of PCR, subtractive hybridization was used to identify differences in gene expression between two tissue samples [29]. However, this method is labor-intensive and usually requires large amounts of RNA which are not readily available from the normal human pituitary or pituitary adenoma samples.

Two newer methods developed to identify differentially expressed genes are differential display (DD) [30] and representational difference analysis (RDA) (see below). For DD, poly(A)-containing RNAs are prepared from two different tissues and converted into cDNA. Each pool of cDNA is amplified with random primers under the same PCR conditions. After amplification, the PCR products from each of the two samples are compared using polyacrydimide gel electrophoresis (PAGE). The presence, absence, or a different intensity of a particular band on the PAGE represents cDNA product which is expressed differently between the two tissue samples. These cDNA products then can be isolated from the gel and cloned for sequence identification.

Using DD, Pei and Melmed compared the gene expression pattern between the normal rat pituitary and a rat GH-secreting tumor cell line GC, and identified a gene, *pituitary tumor transform gene (PTTG)*, whose expression was increased in GC cells compared to the normal rat pituitary [31]. Overexpression of PTTG cDNA in mouse fibroblast 3T3 cells

resulted in *in vitro* cell transformation and *ex vivo* tumor growth, suggesting that *PTTG* is a novel oncogene. Subsequently, human *PTTG* was cloned and its oncogenic function confirmed [32,33]. In the majority of human pituitary adenomas, *PTTG* expression was increased compared to the normal human pituitary [34]. *PTTG* stimulates expression of basic FGF and VEGF, important components in angiogenesis critical for tumor growth [33]. It has been shown that the tumor suppressor p53 suppresses *PTTG* expression and *PTTG* protein physically interacts with p53 [35,36]. *PTTG* has been identified as securin, an important protein functionally involved in chromatin separation [37]; the overexpression of *PTTG* in tumor cells may result in a chromosomal abnormality. Therefore, *PTTG* represents the first oncogene from the pituitary identified by investigation of differences in gene expression between normal pituitary tissue and tumors. Another gene, *BMP-4*, has also been identified by DD to be involved in the pathogenesis of pituitary prolactinomas [38]. It stimulates cell proliferation and expression of the oncogene *c-myc* in human prolactinomas. Interestingly, *BMP-4* has no effect on other types of human pituitary tumors and appears to be lactotroph specific.

The development of microarray techniques has greatly facilitated the investigation of differences in gene expression between normal pituitary and pituitary tumors [39]. This hybridization-based technique allows simultaneous comparison of expression of tens of thousands of genes. Several groups have used this powerful method to compare gene expression between the normal human pituitary and pituitary tumors of various cell origins, and, have identified several genes whose expression is specifically associated with certain tumor types [40-45]. Microarray analysis has also been used to identify expression of microRNAs associated with pituitary tumors [46].

Microarray analyses have revealed many genes that are differentially expressed in the normal human pituitary and pituitary tumors. However, the functional involvement of the majority of these genes in pituitary tumor pathogenesis remains to be determined. A few exceptions to date include *matrix metalloproteinase-9 (MMP-9)* and bone morphogenetic protein- and retinoic acid-inducible neural-specific protein-3 (*BRINP3*). It has been shown that *MMP-9* increases invasion of a pituitary tumor cell line [47] and *BRINP3* induces proliferation, migration, and invasion of pituitary tumor cells [48]. Therefore, further work using microarray analyses will need to be done to identify genes of importance in human pituitary tumor pathogenesis.

Recent data support the importance of epigenetic changes in tumor development. Changes in DNA methylation at specific CpG sites lead to gene activation or silencing, which may in turn result in neoplastic development. By using methylation specific-PCR to isolate and identify novel CpG islands that are differentially methylated in pituitary tumors relative to normal pituitaries, Bahar *et al.* identified a novel *pituitary tumor apoptosis gene (PTAG)* which activates caspase activity and induces apoptosis in mouse pituitary AtT20 cells [49]. Zhu *et al.* reported that down-regulation of fibroblast growth factor receptor 2 (FGFR2) in pituitary tumors was associated with epigenetic silencing through DNA and histone methylation [50], and, down-regulation of FGF signaling via FGFR2 isoform IIIb resulted in increased expression of melanoma-associated antigen A3 (MAGE-A3), which in turn affected histone modification [51].

The investigation of differences in gene expression between normal pituitary and tumors has provided new information about human pituitary tumor pathogenesis. Using representational difference analysis (RDA), we have performed a study focusing on clinically non-functioning pituitary tumors.

Representational Difference Analysis (RDA)

Representational Difference Analysis (RDA) was first developed to analyze the differences in DNA between genomes [52]. Subsequently, it has been modified to identify the differences in cDNA expression between tissue samples (known as cDNA-RDA) by a combination of subtractive hybridization and targeted PCR amplification [53]. We have used this method to analyze the differences between the normal human pituitary and clinically non-functioning pituitary adenomas of a gonadotroph lineage [54]. Briefly, total RNA is extracted from normal human pituitary and clinically non-functioning tumors and converted into separate cDNA pools. A synthetic short oligonucleotide linker is added to the cDNA from the normal pituitary. Next, a small amount of this linker-containing normal pituitary cDNA pool is mixed with a large amount of the tumor cDNA pool. This allows cDNA sequences commonly expressed in both normal and tumor tissues to form a heteroduplex, with one cDNA strand from the normal tissue (which contained the synthetic linker) and the other from tumor tissue (which does not contain the synthetic linker). After hybridization, the sample mix is treated with single-strand-specific nuclease. This enzyme specifically removes the single nucleotide strand corresponding to the synthetic linker in the heteroduplex; the cDNA specifically expressed in the normal pituitary undergoes self-hybridization, thus both strands contain the synthetic linker and are not removed by this nuclease. PCR amplification is then carried out using primers specifically targeted to this synthetic linker. Therefore, normal pituitary specific cDNA is enriched by PCR amplification and subsequently identified by molecular cloning and sequencing.

A number of genes expressed in the normal human pituitary but not in clinically non-functioning tumors were identified by cDNA-RDA (Table 1). Among these genes, the most abundantly expressed gene is *GADD45γ*, a p53-regulated human gene with anti-proliferative activity. RT-PCR confirmed mRNA expression of *GADD45γ* in the normal human pituitary. However, *GADD45γ* is not expressed in the majority of human pituitary tumors of different phenotypes, as well as human pituitary tumor-derived cell lines. Suppression of cell proliferation was observed when *GADD45γ* was expressed in pituitary tumor cell lines. Therefore, *GADD45γ* represents the first human growth inhibitory gene whose expression is lost in the majority of human pituitary tumors [54]. Subsequently it has been shown that loss of *GADD45γ* expression in human pituitary tumors is associated with hypermethylation of this gene in tumors [55].

Compared to microarray analysis, cDNA-RDA is labor-intensive and its ability to identify multiple genes is limited. However, a major advantage of cDNA-RDA is that it can identify novel genes not reported previously; a microarray chip can only contain previously identified or predicted gene sequences. With the completion of human genome sequencing, it has become clear that many alternatively spliced mRNA and non-coding RNA sequences play important physiological functions but cannot be predicted and would be missed in a microarray analysis. Indeed, using cDNA-RDA we have identified a novel non-coding RNA gene, named *Maternally Expressed Gene 3 (MEG3)*, which is hypothesized to be related to the pathogenesis of human clinically non-functioning tumors of a gonadotroph lineage [56].

Maternally Expressed Gene 3 (MEG3): Pituitary Expression and Functions

Maternally Expressed Gene 3 (MEG3) is an imprinted gene expressed only from the maternal allele of the chromosome [57]. Located at chromosome 14q32, it is highly expressed in the pituitary. In addition, MEG3 mRNA is also detected in the brain, placenta, adrenal gland, pancreas, and ovary, suggesting its neuroendocrine-related functions [56].

Complimentary DNA-RDA revealed that MEG3 mRNA is present in the normal human pituitary but absent in clinically non-functioning tumors. Because clinically non-functioning

tumors are mainly derived from gonadotroph cells, while the normal pituitary contains multiple cell types including somatotrophs, lactotrophs, gonadotrophs, corticotrophs, and thyrotrophs, it is conceivable that some genes specifically expressed in cell types other than gonadotrophs will be identified by cDNA-RDA but are not related to tumorigenesis. As shown in Table 1, cDNA-RDA has revealed genes such as the GHRH receptor and prolactin as normal pituitary specific genes when comparing the gene expression differences between the normal human pituitary and clinically non-functioning tumors. Therefore, to confirm that *MEG3* expression is lost in gonadotroph-derived clinically non-functioning tumors, it is important to first establish that *MEG3* is indeed expressed in normal gonadotroph cells. We therefore used combined *in situ* hybridization and immunohistochemistry to detect *MEG3* mRNA in the normal human pituitary, and showed that *MEG3* mRNA was indeed present in normal human gonadotroph cells, co-localized with the gonadotroph-specific hormone FSH [56]. In contrast, *MEG3* mRNA was not detected in clinically non-functioning pituitary tumors by RT-PCR. Interestingly, *MEG3* mRNA was also detected in other cell types of the normal pituitary, including somatotrophs, lactotrophs, corticotrophs, and thyrotrophs. However, in contrast, loss of *MEG3* expression was restricted only to clinically non-functioning tumors; all other types of pituitary tumors, such as GH-, PRL-, ACTH-, and TSH-secreting tumors, express *MEG3* RNA (Figure 1) [58]. Therefore, *MEG3* is thus far the only human gene specifically associated with gonadotroph derived clinically non-functioning pituitary tumors.

To understand the mechanism for the loss of *MEG3* expression in clinically non-functioning tumors, we have examined *MEG3* genomic structure in these tumors [59]. No gene deletion or mutation was found in such tumors. However, increased DNA methylation was found in the 5'-flanking region of the *MEG3* gene in tumors compared to that in the normal pituitary. The increased CpG methylation sites were specifically located at two positions: one is the TATA-containing promoter; the other is an enhancer. Furthermore, treatment of human cancer cells with a de-methylating agent resulted in expression of *MEG3* in these cells [59]. Clearly, DNA methylation within the functional regulatory regions of *MEG3* gene is associated with loss of *MEG3* expression in tumor cells.

To link *MEG3* with tumor pathogenesis, we examined the anti-proliferative function of *MEG3* using multiple approaches, including classical colony formation assays, direct cell counting, and BrdU incorporation assays. In every assay, *MEG3* consistently showed a strong growth-inhibitory function; suppressing proliferation of different types of human tumor cells by 60-90% (Figure 2) [56,60]. To investigate the mechanism by which *MEG3* suppresses tumor cell growth, we examined the potential relationship between *MEG3* and p53, one of the most important tumor suppressors. We found that in reporter assays, *MEG3* stimulated p53-mediated transcriptional activation. Expression of *MEG3* resulted in cellular accumulation of p53 protein; decrease in the protein level of MDM2, the negative regulator of p53 (Figure 3); and activation of p53 downstream target genes in a selective manner. For example, the expression of *p21^{CIP1}*, one of the most well-known p53 target genes, was not affected by *MEG3*. Instead, expression of *GDF15*, a TGF- β family member with an anti-proliferative activity, was increased by *MEG3* [60]. The mechanism whereby *MEG3* uncouples p53 from p21^{CIP1} is currently unknown. Activation of p53 can be achieved by its modification via phosphorylation and acetylation, or by physical interaction with other proteins, such as p14^{ARF}. Upstream signals modify p53 at specific sites, resulting in activated p53 capable of activating specific target genes. It is possible that modification of p53 at specific sites induced by *MEG3* or direct interaction between *MEG3* and p53 results in selective activation of p53 target genes. Further investigation is required to clarify these mechanisms.

Although the function of tumor suppressor p53 is clearly stimulated by MEG3, MEG3 is also capable of suppressing cell proliferation in the absence of p53, indicating that it can function in both p53-dependent and -independent manner. Interestingly, in the absence of p53, the tumor suppressor Rb is required for MEG3-mediated growth suppression [60].

Taking together, our data suggest that MEG3 may functionally interact with both p53 and Rb to control cell proliferation. Figure 4 represents a conceptual framework of MEG3 in pituitary tumorigenesis. MEG3 may activate p53 by either suppressing the negative regulator MDM2 or directly interacting with p53 or affecting p53 modification, resulting in selective activation of p53 downstream targets such as GDF-15 and other yet-to-be-identified proteins with anti-proliferative and tumor suppressive functions. MEG3 may also activate Rb, independent of p53, by direct RNA-protein interaction, by regulating Rb phosphorylation, or, by activation of the positive regulator p16^{INK4a}, thereby suppressing cell proliferation and tumor formation. Defects in the Rb pathway have been found in human pituitary tumors [61]; in particular, loss of p16^{INK4a} expression is frequent in clinically non-functioning tumors (up to 70%), presumably caused by hypermethylation of this gene; but is infrequent in hormone-secreting tumors (less than 20%) [61-63]. Considered in the context of our finding of exclusive loss of MEG3 expression in clinically non-functioning tumors, these data strongly suggest a potential link between MEG3 and p16^{INK4a}.

It was hypothesized that *MEG3* gene products may function as a non-coding RNA. Sequence analysis revealed that at a full length of approximately 1.7 K nucleotides, MEG3 cDNA only contained several small open reading frames (ORF), all potentially encoding peptides less than 100 amino acids. Furthermore, there was no Kozak sequence for each ORF; so it was unlikely that these ORFs would be translated into peptides. To prove this hypothesis, we generated several MEG3 mutants, in which each or every ORF was destroyed by a point mutation and became untranslatable. When these MEG3 mutants were used in functional assays to stimulate p53-mediated transactivation and to suppress DNA synthesis, we found that all the mutants were fully functional, indicating that peptide translation was not required for MEG3 function. In contrast, if a promoterless vector for MEG3 expression was used, no MEG3 function was observed. Taken together, these results clearly demonstrate that the biological functions of MEG3 require gene transcription to generate a RNA molecule, but do not require RNA translation to generate a protein. Therefore, our experimental results have proven that the *MEG3* gene product is indeed a non-coding RNA [60].

The finding of a large non-coding RNA with such profound biological functions is intriguing. There is no sequence homology between MEG3 RNA and the RNA sequence of its putative targets such as p53 and Rb. A microRNA, mir770, has been reported located within the introns of *MEG3* gene ([http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=retrieve&dopt=full_report&list_uids=768222&log=\\$=genesensor5&logdbfrom=pubmed](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=retrieve&dopt=full_report&list_uids=768222&log=$=genesensor5&logdbfrom=pubmed)). However, the expression and functions of this microRNA in the pituitary and other tumors have not been examined. Likely the functions of MEG3 do not depend on this microRNA, as it is not included in the MEG3 RNA products.

Lost of imprinting and human pituitary tumor pathogenesis

Genomic imprinting describes a phenomenon in which the expression of a gene depends upon its parental origin. Imprinted genes are usually clustered on a chromosome to form imprinted foci. Several such foci have been identified in humans, such as the *delta like 1* homologue (*DLK1*)/*MEG3* locus on chromosome 14q32 [57,64], *H19/Insulin like growth factor 2* (*IGF2*) locus on chromosome 11p15 [65,66], and the *Prader-Willi syndrome*/

Angelman syndrome (PWS/AS) locus on chromosome 11q11-q13 [67]. Each of these loci contains multiple maternally and paternally expressed genes. Gene expression in these imprinted loci is tightly regulated by imprinting control regions (ICRs), which are genetic cis elements functioning as insulators or activators. Genetic or epigenetic alterations in ICRs usually lead to silencing of the normally expressed allele, or activation of the normally silenced allele of an imprinted gene, which is referred to as loss of imprinting (LOI). LOI has been linked to the development of many human diseases including tumors. For example, LOI of the *H19/IGF2* locus has been found in many types of human cancers, including Wilms' tumor [68,69], lung cancer [70,71], colorectal cancer [72] and hepatoblastoma [73,74]. Because LOI usually results in elevated expression of IGF2 and/or reduced expression of H19, it is likely that the tumorigenicity of LOI of *H19/IGF2* locus is attributable to the tumor suppression activity of non-coding RNA H19 [75] and growth promoting activity of IGF2 [76]. Recently, it was also suggested that the *H19/IGF2* locus acts as a tumor suppressor *in vivo* [77].

The *DLK1/MEG3* locus contains multi maternally expressed genes including *MEG3*, *anti-Rtl1*, *Rian*, *Mirg*, numerous small nucleolar RNAs and micro RNAs; and paternally expressed genes including *DLK1*, *Rtl1* and *Dio3*. Interestingly, all maternally expressed genes are non-coding RNAs, while all paternally expressed genes are protein coding genes. The ICR of the *DLK1/MEG3* locus is a DNA element, known as the intergenic differentially methylated region (IG-DMR), which is methylated on the paternal chromosome [78,79]. The unmethylated IG-DMR on the maternal chromosome acts as an activator positively regulating expression of maternally expressed genes [79]. Data from our laboratory and others indicate that LOI of *DLK1/MEG3* locus is linked to a number of human tumors including human pituitary tumors. Astuti *et al.* reported that *MEG3* expression is lost in primary neuroblastoma, pheochromocytoma and Wilms' tumors [80]. As previously discussed, we have found that *MEG3* expression is silenced in virtually all gonadotroph-derived clinically non-functioning pituitary adenomas, but not in functioning tumors [56,58,59]. Methylation in the IG-DMR and *MEG3* promoter region was found to be increased in a percentage of tumors, but not in all tumors examined [58,59], suggesting that inactivation of the ICR and the promoter is only one of mechanisms by which LOI occurs in those tumors. However, other mechanisms accounting for LOI of the *DLK1/MEG3* locus in non-functioning pituitary tumors remain elusive. Evidence indicates that LOI of both *H19* and *IGF2* in the *H19/IGF2* locus contributes to tumor development [75,76]. The *DLK1/MEG3* locus consists of many maternally expressed non-coding RNAs and paternally expressed protein coding genes. This raises the question of whether those genes proximal to *MEG3* are also involved in the development of these pituitary tumors. One of the potential candidate genes whose expression may be affected by LOI of *MEG3* is *DLK1*. *DLK1*, also known as *Pref-1*, functions to inhibit adipocyte differentiation [81]. Elevated expression of *DLK1* has been found in gliomas and ectopic expression of *DLK1* stimulates proliferation of glioblastoma cells lines [82]. Recently, *DLK1* expression was found to be activated by hPTTG and was thought to mediate differentiation inhibition by hPTTG [83]. More importantly, Kim *et al.* reported that *DLK1* expression at high levels inhibits differentiation and enhances tumorigenic potentials in tumor cells [84]. These data indicate that *DLK1* has oncogenic potential. Considering the mounting data demonstrating the importance of LOI in development of other human neoplasms, it is reasonable to predict that LOI of *DLK1/MEG3* locus may play an important role in the pathogenesis of human pituitary tumors. The *DLK1/MEG3* locus consists of numerous imprinted genes, many of which are non-coding RNAs. Its imprinting regulation is complex and is not yet fully understood. Therefore, the challenge is to identify which gene has lost its imprinting, the mechanisms by which the LOI occurs and how the identified gene contributes to tumor development.

Conclusions

In the past decade, the development of several novel techniques has made it possible to investigate and compare the global profile of gene expression in tumor cells and normal tissues. Many researchers worldwide have applied these new techniques to the investigation of human pituitary tumors and significant progress has been made in this field. Many new target genes have been identified as candidates associated with human pituitary tumorigenesis, and the functions and regulation of these genes are being actively investigated. As one such candidate, *MEG3* is unique because this gene does not encode a protein but a large non-coding RNA. To understand how a large non-coding RNA molecule is involved in the control of cell proliferation, it will be important to investigate its molecular structure, its functional interaction with other important mediators in tumor biology such as p53 and Rb, and the relationship between its imprinting control and tumor development. Animal models such as a *MEG3* knockout and/or transgenic expression will be critical to reveal other important physiological functions of this novel RNA. Further investigation of *MEG3* has the potential to introduce a new concept in our understanding of cell growth control and the development of clinically nonfunctioning pituitary tumors.

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Fig 1. RT-PCR showing MEG3 RNA expression in all cases of functioning pituitary adenomas (A, GH-secreting tumors; B, PRL-secreting tumors; and C, ACTH-secreting tumors) and normal human pituitaries (D). Some prolactinomas have a weaker expression (in two cases) (B). In contrast, no MEG3 RNA is detected in any of the 17 clinically non-functioning tumors (NFT) (E). NP: positive control with normal pituitary cDNA; C: negative control without cDNA template. (Copyright 2008, The Endocrine Society)

Fig 2.

MEG3 suppresses tumor cell growth. (A) H4 cells were transfected with the blank vector pCI-neo (control) or expression vector for *LacZ*, *MEG3*, or *GADD45-γ*. After 2 weeks of neomycin selection, the plates were fixed and stained with crystal violet solution. (B) Viable colonies of HeLa cells, MCF-7, and H4 cells in similar experiments, were counted and normalized to control. The data are represented as mean $\pm$ SD for counts from at least three independent experiments. (Copyright 2003, The Endocrine Society)

Fig 3.

MEG3 activates p53 and downregulates MDM2. HCT116 cells were transfected with constructs expressing p14^{ARF} or MEG3 as indicated. Expression of p53 and MDM2 were detected by Western blotting. β -Actin was probed as an equal protein loading control. (Copyright 2007, The American Society for Biochemistry and Molecular Biology)

Figure 4.

MEG3 interacts with both p53 and Rb pathways. In the p53 pathway, MEG3 may activate p53 directly by RNA-protein interaction or indirectly by suppressing MDM2, resulting in selective activation of p53 downstream targets such as GDF-15 with both anti-proliferative and tumor suppressive functions. In the Rb pathway, MEG3 may activate Rb directly by RNA-protein interactions or indirectly by activating the positive regulator p16^{INK4a}, which in turn activates Rb pathway to suppress cell proliferation and tumor formation.

Table 1

Normal Pituitary Specific cDNAs identified by cDNA-RDA

Name of cDNA	Number of appearance
GADD45- γ (a.k.a. cytokine responsive protein CR6)	81
histone deacetylase 6	51
unknown cDNA X2, partially homologous to MEG3	49
delta (Drosophila) like 1 (DLK1)	46
unknown cDNA X6, partially homologous to mouse Sarp1	16
ribosomal protein S15	11
GHRH receptor	9
Pleckstrin and Sec7 domain protein	6
Unknown cDNA X1	6
adaptin β 1	3
syntaxin	3
phosphatidylethanolamine N-methyltransferase	3
small cytoplasmic RNA 7SL	3
keratin 18	2
collagen IX- α 2	2
cleavage and polyA specificity factor	2
unkown cDNA X8, partially homologous to human DNA sequence from chromosome 16p13.3	2
prolactin	1
α -1-antichymotrypsin	1
Kunitz-type protease inhibitor	1
T-cell immune regulator-1	1
cholesterol esterase	1
granulin	1
proline dehydrogenase	1
human clone RG161A02	1
unknown cDNA X7, partially homologous to human chromosome 17 clone hRPK.180_P_8	1
unknown cDNA X9, partially homologous to phosphatidylinositol 4-kinase	1
unknown cDNA X10, partially homologous to neuronal PAS domain protein	1
unknown cDNA X3	1
unknown cDNA X4	1
unknown cDNA X5	1
Total number of sequences analyzed:	309

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