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Short Communication

Translocation, Deletion/Amplification, and Expression of *HMGIC* and *MDM2* in a Carcinoma ex Pleomorphic Adenoma

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Carcinoma ex pleomorphic adenoma (CexPA) is a carcinoma developing within a pre-existing benign pleomorphic adenoma (PA). Here we describe the identification and characterization of a series of genetic events leading to translocation, deletion/amplification, and overexpression of the *HMGIC* and *MDM2* genes in a CexPA at an early stage of development. The tumor had a pseudodiploid stemline karyotype with a del(5)(q22–23q32–33) and a t(10;12)(p15;q14–15). In addition, there were several sidelines with double minute chromosomes (dmin) or homogeneously staining regions (hsr). Fluorescence in situ hybridization (FISH) mapping revealed that the 12q14–15 breakpoint was located centromeric to *HMGIC* and that the entire gene was juxtaposed to the der(10) chromosome. Detailed analysis of cells with dmin and hsr revealed that *HMGIC* and *MDM2* were deleted from the der(10) and that the dmin and hsr were strongly positive for both genes. Southern blot analysis confirmed that both *HMGIC* and *MDM2* were amplified and that no gross rearrangements of the genes had occurred. Immunostaining revealed that the HMGIC protein was highly overexpressed particularly in the large polymorphic cells within the carcinomatous part of the tumor. These findings suggest that amplification and overexpression of *HMGIC* and possibly *MDM2* might be important genetic events that may contribute to malignant transformation of benign PA. *(Am J Pathol 2002, 160:433–440)*

The pleomorphic adenoma (PA) is the most common type of salivary gland neoplasm. It is usually a benign, slow-growing tumor originating from the minor and major salivary glands.[1] Microscopically, PA show a wide morphological spectrum with mainly epithelial and myoepithelial cells forming a variety of patterns in a mucoid, myxoid, or chondroid matrix. Occasionally, these normally benign tumors may undergo malignant transformation. The frequency by which this occurs varies in different series from about 2 to 23%. For example, in the AFIP series of 326 carcinoma ex pleomorphic adenoma (CexPA) cases, they represented 4.5% of all PA and 6.5% of all malignant salivary gland tumors.[2] The incidence of malignant transformation increases with the preoperative duration of the tumors.[3] CexPA is usually an aggressive tumor. Almost one-half of the patients develop recurrences, and approximately one-third of the patients with parotid tumors develop metastases.

Cytogenetic information about the chromosomal pattern in CexPA is scarce; only 14 cases have so far been analyzed.[4–14] In contrast, our knowledge about the cytogenetics of benign PA is comprehensive. Karyotypic data are available for almost 500 cases.[15–18] About 70% of the tumors have abnormal karyotypes. Four major cytogenetic subgroups have been identified, ie, tumors with rearrangements involving 8q12 (39%), tumors with rearrangements of 12q14–15 (8%), tumors with sporadic, clonal changes not involving 8q12 or 12q14–15 (23%), and tumors with an apparently normal karyotype (30%).

Recently, we identified the genes consistently rearranged in PA with 8q12 and 12q14–15 abnormalities. The target gene in 8q12 is *PLAG1*, a developmentally regulated zinc finger gene.[19–21] The translocations result in

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promoter swapping/substitution between *PLAG1* and a ubiquitously expressed translocation partner gene (eg, *CTNNB1*, *LIFR,* or *SII*), leading to activation of *PLAG1* expression. The breakpoints invariably occur in the 5′ noncoding regions of both the target gene and the promoter donor genes. The target gene in adenomas with rearrangements of 12q14–15 is the high mobility group protein gene, *HMGIC*.[22–24] This gene is also rearranged in a variety of mesenchymal tumors.[22,25] *HMGIC* encodes an architectural transcription factor that promotes activation of gene expression by modulating the conformation of DNA.[26] The protein has three DNA-binding domains (AT-hook motifs) that bind to the minor groove of AT-rich DNA.[27] The majority of breakpoints in *HMGIC* occur within the third large intron, resulting in separation of the DNA-binding domains from the highly acidic, carboxy-terminal domain. Several translocation partner genes have been identified, including *ALDH2*, *LPP*, *LHFP*, *RAD51B*, *COX6C*, *HEI10*, *FHIT*, and *NF1B.*[23,24,28–33] The two latter are fusion partners identified in PA with t(3;12) and ins(9;12).[23,24] Since no common functional domain so far has been identified among the translocation partners, the critical event seems to be the separation of the DNA-binding domains from the acidic carboxy-terminal tail of HMGIC.[23]

We report here extensive molecular cytogenetic characterization of a CexPA at an early stage of development. Detailed analysis revealed a t(10;12)(p15;q15) translocation with a 12q breakpoint 5′ of *HMGIC* and translocation of the entire gene to the 10p+ marker chromosome followed by deletion/amplification of a segment containing *HMGIC* and *MDM2* from this marker. The amplified sequences were mapped to double minute chromosomes (dmin) and homogeneously staining regions (hsr). These findings suggest that amplification of *HMGIC* and *MDM2* might be important genetic events in the malignant transformation of benign PA.

Materials and Methods

Tumor Material and Cytogenetic Analysis

Fresh tumor tissue was obtained from a 35-year-old woman who had a several months history of a tumor in the left parotid gland. The tumor, which measured 11 × 18 × 20 mm, was removed with tumor-free margins by a superficial parotidectomi. Macroscopically, the tumor was circumscribed and had solid, gray-white cut surfaces. Microscopic examination revealed a cell-rich salivary gland tumor with occasional foci characteristic of PA with monomorphic tumor cells growing in strands and nests in a hyalinized stroma (Figure 1,A). The overall histological appearance was, however, that of a carcinoma with pronounced cellular polymorphism (Figure 1, B and C). Certain solid areas were comprised of small cells with minimal cytoplasm, others of large polymorphic, cytoplasm-rich cells. There was a pronounced cellular atypia with enlarged, polymorphic, and hyperchromatic nuclei containing prominent nucleoli (Figure 1, B and C). Serial sections of the tumor specimen revealed areas of microinvasion with growth of tumor nests in a vascularized stroma (Figure 1D). Immunostaining revealed that the polymorphic tumor cells had a strong nuclear positivity for the HMGIC oncoprotein (Figure 1E) (see Results). Occasional mitotic figures were observed in the carcinomatous areas. Focally, the tumor showed a moderate proliferative activity as judged by immunostaining of Ki-67 (Figure 1F). Immunostains for cytokeratin (CAM 5.2), vimentin and S100 were also positive in parts of the tumor.

The overall morphological picture of the tumor with high cellularity, pronounced cellular polymorphism, and microinvasion together with the results of the immunostains were considered compatible with the diagnosis of a CexPA at an early stage of development. Subsequently, a total parotidectomi was performed. Histopathological examination revealed no signs of tumor growth in the resected specimens. The patient received no adjuvant treatment. Three years postoperatively there were no signs of local recurrences or metastases.

Primary cultures were established from a fresh, unfixed specimen of the primary tumor as previously described.[34] Chromosome preparations were made from exponentially growing primary cultures and these were subsequently G-banded and analyzed using standard procedures.

FISH and Spectral Karyotype Analyses

Metaphase spreads used for FISH were prepared from cells stored in fixative at −20°C. The following probes were used: whole chromosome painting probes specific for chromosomes 5, 9, 10, 12, and 13 (Vysis, Inc., Downers Grove, IL); CEPH YACs 975B8 (*SAS/CDK4*); 811A7 (*MDM2*); 452E1 (*HMGIC*); the LL12NCO1-derived cosmid clones 142H1 and 27E12 (containing exons 1–2 and 4–5, respectively, of *HMGIC*);[22] the microdissection library ML12q13–15 (specific for the 12q13–15 segment); and the PAC-clones PAC233 and PAC235 (*PLAG1*). DNAs were either amplified by InterAlu-PCR and labeled with biotin-16-dUTP (Roche Diagnostic, Basel, Switzerland) or labeled with biotin-16-dUTP (Roche Diagnostic), and subsequently cohybridized with α-satellite probes for chromosomes 8, 9, 10, 12, and 13 (Appligene Oncor, Qbiogene, Carlsbad, CA) in different combinations. Hybridization and probe detection were as previously described.[35] Chromosomes were counter-stained with 4′,6′-diamidino-2′-phenylindole dihydrochloride (DAPI). FISH analysis of formalin-fixed, paraffin-embedded tissue sections were performed using the tissue conversion kit S1337-TC and *in situ* hybridization kit S1340 (Appligene Oncor). The sections were counter-stained with propidium iodide. Fluorescence signals were digitalized, processed, and analyzed using the PowerGene FISH image analysis system (Applied Imaging International Ltd., Newcastle-upon-Tyne, UK).

Spectral karyotype (SKY) analysis was performed using the SkyPaint probe kit which consists of a cocktail of 24 differentially labeled chromosome specific painting probes (ASI-Applied Spectral Imaging Ltd., Migdal Ha'Emek, Israel). The conditions for pretreatment, hybrid-

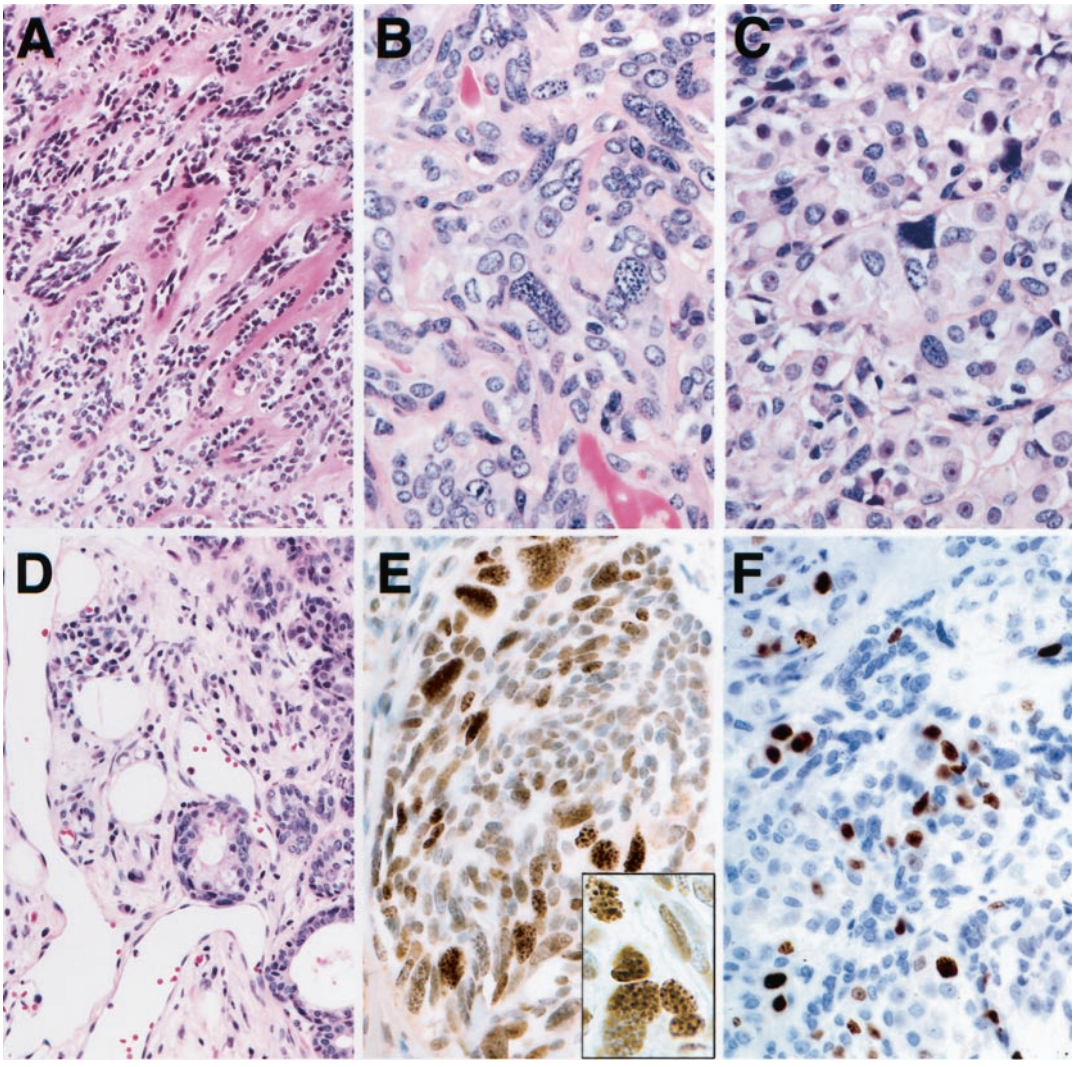

Figure 1. Morphology of the carcinoma ex pleomorphic adenoma. **A:** Residual PA with mainly nonluminal tumor cells in a hyaline stroma. **B** and **C:** Details of the carcinomatous component of the tumor. Note the pronounced cellular atypia with enlarged, polymorphic and hyperchromatic nuclei and sometimes prominent nucleoli. **D:** Microinvasion with growth of nests of tumor cells in close proximity to thin-walled vessels. **E:** Strong nuclear immunoreactivity for the HMGIC oncoprotein. The highest expression is seen in the large polymorphic nuclei. Note the localization of the protein to granular nuclear structures (**inset**). **F:** Positive nuclear immunoreactivity for Ki-67.

ization, posthybridization washes, detection, and analyses were as previously described[36] and as recommended by the manufacturer.

Immunohistochemical Analysis

Tissue sections were processed according to the avidin-biotin complex (ABC) method. Briefly, sections were deparaffinized, treated in a microwave oven and exposed to hydrogen peroxidase. A polyclonal HMGIC antibody was obtained by immunizing rabbits with a peptide corresponding to a sequence in the N-terminal part of the human HMGIC protein (SARGEGAGQPSTSA) (GSAB4, dilution 1:25; Innovagen AB, Lund, Sweden). The antiserum was affinity purified using the same peptide. The specificity of the antibody was confirmed by analysis of known HMGIC positive and negative PA.[21] The MDM2 protein was detected by two mouse monoclonal antibodies; clone IF2 (dilution 4 μg/ml; CN Biosciences, Inc./Calbiochem, Darmstadt, Germany) recognizes an epitope within amino acid residues 26–169 of the human MDM2 protein and clone 1B10 (dilution 1:50; Novocastra Laboratories Ltd., Newcastle-upon-Tyne, UK) recognizes an epitope in the carboxy-terminal portion of the MDM2 protein. Other primary antibodies used for immunohistochemistry were: TP53 (DO-7, dilution 1:200; DAKO A/F, Glostrup, Denmark), Ki-67 (MIB-1, dilution 1:100; DAKO), cytokeratin (CAM 5.2, dilution 1:10; Becton Dickinson, Franklin Lakes, NJ), vimentin (dilution 1:400; DAKO), and S100 (dilution 1:1000; DAKO). Control sections were incubated identically, except for the primary antibodies, which were replaced by bovine serum in TBS.

Southern Blot Analysis

Four μg of normal and tumor DNAs were digested with *Hind*III, electrophoresed in a 0.8% agarose gel in 0.5X

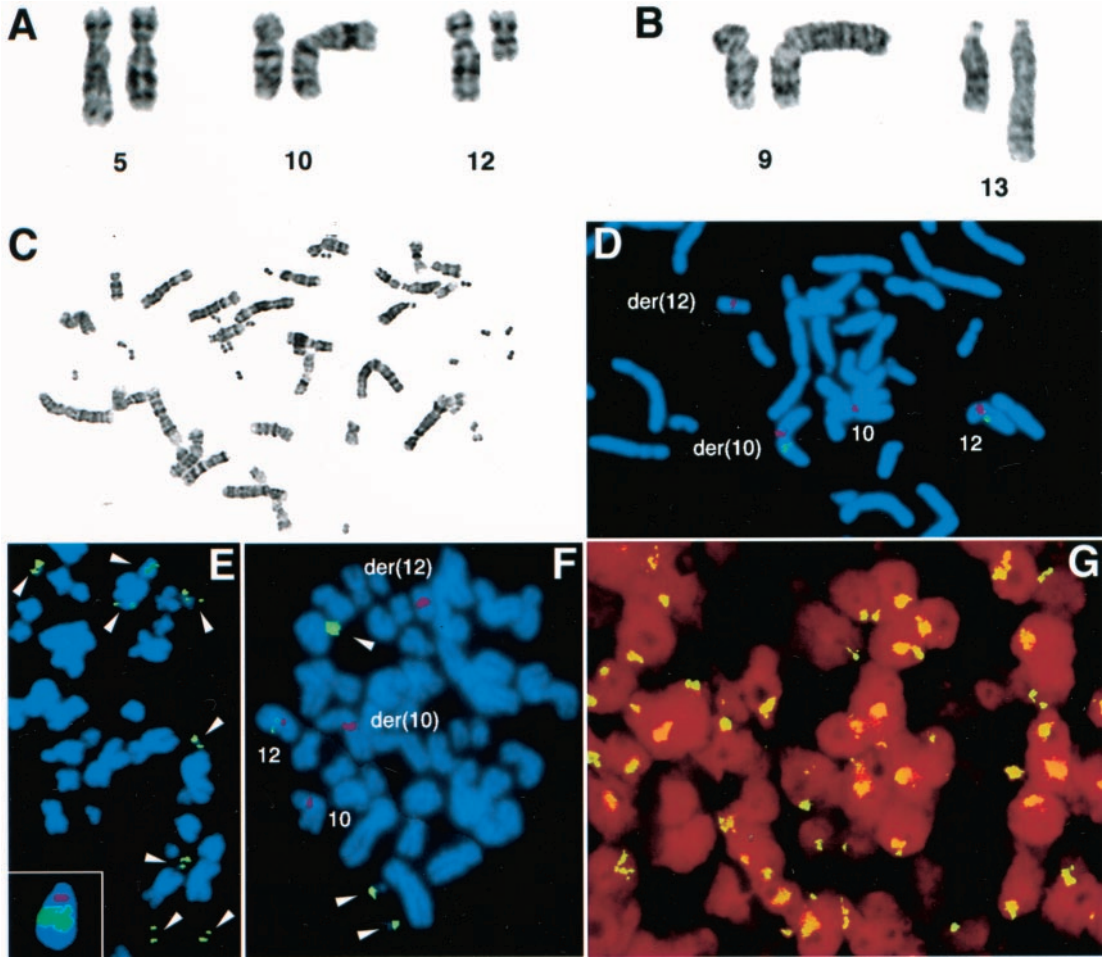

Figure 2. A: Partial G-banded karyotype of the Cet PA tumor showing the del(5)(q22–23q31–32) and the t(10;12)(p15;q14–15) translocation. **B:** G-banded chromosome 9 and 13 homologues with hsr's in 9p22–24 and 13q14. **C:** Partial G-banded metaphase with dmin. **D:** FISH analyses of the 12q14–15 breakpoint. YAC 452E1 (*HMGIC*; green signal) was cohybridized with α-satellite probes for chromosomes 10 and 12 (red signals). Green hybridization signals were noted on the normal chromosome 12 and the der(10), indicating that the breakpoint is proximal to *HMGIC*. **E:** Hybridization with cosmid 142H1 (green signal) showed that the dmin (**arrowheads**) and hsr(13) (**inset**) were strongly positive for *HMGIC*. **F:** Cohybridization of YAC 811A7 (*MDM2*; green signal) and α-satellite probes for chromosomes 10 and 12 (red signals) revealed that *MDM2* was deleted from the der(10) and that the dmin (**arrowheads**) were strongly positive for *MDM2*. **G:** FISH analyses of tissue sections using YAC 811A7 (*MDM2*; green/yellow signal) revealed strong nuclear hybridization of a large proportion of the cells in the malignant part of the tumor. Nuclei are counter-stained in red with propidium iodide.

tris-borate-ethylenediamine-tetra-acetic acid (TBE) buffer, and transferred to a Hybond N^+ membrane. The *MDM2* probe used was a 600-bp fragment corresponding to nucleotides 53 to 653 of the human cDNA (GenBank accession number Z12020). Two *HMGIC* probes were used: an 83-bp fragment derived from the 5′ non-translated region which corresponds to nucleotides 1–83 of the *HMGIC* cDNA (GenBank accession number Z31595) and a 225-bp fragment derived from the 3′ flanking region corresponding to nucleotides 18–242 of STS 12-RM133 (GenBank accession number U27137). As control for equal loading of DNA a 506-bp probe corresponding to the entire coding region of the *CHOP* gene in 12q13 (nucleotides 75–581; GenBank accession number X67083) was used. Probes were labeled with α-[^{32}P]dCTP by random priming or by specific primers.

TP53 Mutation Analysis

Genomic DNA was isolated from tumor cells using standard methods. Exons 4–9 of the *TP53* gene were amplified as previously described.[37] For DNA sequence analysis, 40 μl of the PCR products were denatured and the strands were separated using streptavidin-coated magnetic beads (Dynabeads M-280, Dynal, Norway). Solid support sequencing was performed using the Sequenase Version 2.0 (US Biochemical, Cleveland, OH). Samples were run on 6% denaturating polyacrylamide gels for 1.5 to 4.5 hours and subsequently exposed to x-ray films.

Results

Cytogenetic, SKY, and FISH Analyses

Cytogenetic analysis of short-term cultured cells revealed that the tumor had the stemline karyotype 46, XX, del(5)(q22–23q32–33), t(10;12)(p15;q15)[11] (Figure 2,A). There were also four closely related sidelines with the karyotypes 46, XX, del(5)(q22–23q32–33), t(10;12)(p15;q15),1–34dmin[13]/46, XX, del(5)(q22–23q32–

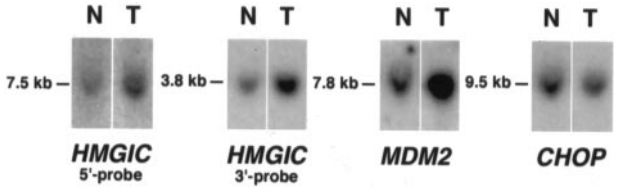

Figure 3. Southern blot analysis of normal (N) and tumor (T) DNAs showing amplification of the *HMGIC* and *MDM2* genes. DNAs were digested with *Hind*III, electrophoresed, transferred, and sequentially hybridized with probes for *HMGIC* (5′ and 3′ sequences), *MDM2*, and *CHOP* (as control). Fragment sizes are indicated to the **left**.

33), t(10;12)(p15;q15), hsr(13)(q14)[5]/46, XX, t(X;6)(p11.2;q27), del(5)(q22–23q32–33), t(10;12)(p15;q15)[5]/46, XX, del(5)(q22–23q32–33), hsr(9)(p22–24), t(10;12)(p15;q15)[3] (Figure 2, B and C). In addition, there were seven cells with a normal female karyotype. To confirm the presence of the del(5), t(10;12), and t(X;6) and to search for possible cryptic rearrangements, we also performed SKY analysis. Detailed analysis of the SKY and DAPI-band images from 5 metaphases corroborated the cytogenetic observations. No cryptic rearrangements were detected. Analysis of one cell with dmin suggested that the dmin contained chromosome 12 sequences. FISH analysis using painting probes for chromosomes 5, 9, 10, 12, and 13 confirmed that both the dmin and the hsr(9) and hsr(13) were derived from chromosome 12.

To further map the chromosome 12q15 breakpoint in relation to *HMGIC*, *SAS*, *CDK4*, and *MDM2* (all located at 12q14–15) we used the microdissection library ML12q13–15 as well as YAC and cosmid clones containing these genes. Detailed mapping revealed that the 12q15 breakpoint was located proximal to *HMGIC*, but distal to the *SAS* and *CDK4* genes, resulting in translocation of the entire *HMGIC* gene to the der(10) marker (Figure 2D). Hybridization with cosmid clones corresponding to the 5′- and 3′-parts of *HMGIC* confirmed that the gene was not split by the translocation. The dmin and hsr were strongly positive for *HMGIC* (both 5′ and 3′ sequences) (Figure 2E) and *MDM2* (located distal to *HMGIC*) (Figure 2F), but not for *SAS* or *CDK4*, indicating that *HMGIC* and *MDM2* are coamplified in the dmin and hsr. Interestingly, in the metaphases containing dmin or hsr, no signals could be observed from the *HMGIC* and *MDM2* containing YACs on the der(10) marker (Figure 2F). This indicates that both genes were deleted from this marker and that the deleted segment originally was not eliminated but retained as dmin or as an hsr. FISH analysis of the *PLAG1* locus at 8q12 revealed signals only on the two chromosome 8 homologues, indicating that *PLAG1* is not rearranged.

Amplification and Expression of HMGIC and MDM2

Southern blot analysis of tumor DNA showed that both *HMGIC* and *MDM2* were amplified compared to normal control DNA (Figure 3). Hybridization with probes corresponding to the 5′ and 3′ parts of *HMGIC*, respectively, revealed that the entire gene was amplified. No rearrangement of the *HMGIC* or *MDM2* genes was observed. Control hybridization with a *CHOP* probe showed that *CHOP*, which is located centromeric to *HMGIC* in band 12q13, was not amplified.

FISH analysis of tissue sections from the tumor using *HMGIC* and *MDM2* specific YACs revealed strong hybridization signals in about 25 to 50% of the tumor cells (Figure 2G). To study whether the amplified genes were expressed we used immunohistochemistry and HMGIC and MDM2 specific antibodies. Nuclear expression of the HMGIC protein was detected in about 25 to 50% of the tumor cells (Figure 1E). About half of these cells expressed high levels of HMGIC. The HMGIC protein localized to granular, nuclear structures similar in size and appearance to the so-called PML nuclear bodies.[38] A similar, but less pronounced pattern, could also be seen in the other HMGIC positive cells. The location of the HMGIC positive cells largely coincided with the location of the cells with strong FISH signals for HMGIC and MDM2. The strongest HMGIC staining was observed in large, polymorphic atypical nuclei (Figure 1E). Control staining of tissue sections from a PA with known overexpression of an *HMGIC-NF1B* fusion transcript due to an ins(9;12)(p23;q12q15)[24] revealed an evenly distributed nuclear expression of the protein in the majority of the tumor cells (not shown). No cells with very high expression levels were observed. Staining of sections from a PA without rearrangements of *HMGIC* revealed no nuclear staining in any of the tumor cells. Immunostaining for MDM2 revealed a few scattered positive cells. Similar results were obtained with both antibodies used. Immunostaining for TP53 protein was negative. Nucleotide sequence analysis of the *TP53* gene revealed no mutations in exons 4 to 9.

Discussion

In this communication we describe the identification and characterization of a series of genetic events leading to translocation, deletion/amplification, and overexpression of the *HMGIC* gene in a case of CexPA. The carcinoma, which was at a relatively early stage of development, had a stemline karyotype with a del(5)(q22–23q32–33) and a t(10;12)(p15;q15) as the sole cytogenetic abnormalities. Since translocations with breakpoints in 12q14–15 are characteristic of a subgroup of PA,[15–17] it is likely that the carcinoma originated from a PA belonging to this subgroup. FISH mapping revealed that the 12q breakpoint was located telomeric to *CDK4* and *SAS* and centromeric to *HMGIC* and that the entire gene was juxtaposed to the der(10) chromosome. Although most translocation breakpoints in 12q15 are located within introns 3 and 4 of *HMGIC*, there are several cases of uterine leiomyomas[39] as well as single cases of PA[40] reported with breakpoints located either proximal or distal to *HMGIC*. For these cases as well as for the present case, a mechanism of deregulation not involving the generation of an *HMGIC* containing fusion transcript must be considered.

About one-half of the tumor cells carrying the t(10;12) translocation had also dmin or an hsr. FISH analysis of these cells revealed that the *HMGIC* and *MDM2* genes were deleted from the der(10) chromosome and that the dmin and hsr were strongly positive for both genes. Detailed analysis showed that both genes were coamplified in the same dmin and hsr. Southern blot analysis confirmed that *HMGIC* and *MDM2* were amplified and that no gross rearrangements of the genes had occurred. The mechanisms of gene amplification and of the generation of dmin and hsr in tumor cells are still somewhat obscure. Our findings of a series of genetic events leading to gene amplification in CexPA are in line with the proposed deletion-plus-episome model in which recombination leads to chromosomal deletion and episome formation.[41] The episomes may enlarge by homologous recombination and replication and as a consequence become visible as extrachromosomal dmin. The dmin can subsequently integrate at random chromosomal sites to generate an hsr.[42]

The consequences of amplification of *HMGIC* and *MDM2* on gene expression were evaluated by immunohistochemistry using HMGIC and MDM2 specific antibodies. Very high expression levels of HMGIC was found particularly in the large polymorphic nuclei within the carcinomatous part of the tumor, indicating that these cells contain the amplified *HMGIC* sequences. In contrast, the expression level of MDM2 was much lower. The reason for this discrepancy is not known. Since multiple forms of MDM2 proteins exist expressing different epitopes it could be that the monoclonal antibodies used in this study failed to detect the particular epitopes expressed in the tumor. Alternatively, the amplified *MDM2* sequences were not expressed. In a previous study of soft tissue sarcomas only 6 of 11 cases with *MDM2* amplification expressed the MDM2 protein.[43] FISH analysis of paraffin sections of the present tumor confirmed that the amplified *HMGIC* and *MDM2* sequences preferentially were located in the carcinomatous part of the tumor.

Cytogenetic data on CexPA is scarce. Including the present case, karyotypic information is available only from 15 cases (13 salivary gland and two lacrimal gland tumors).[4–14] Eight of these have shown rearrangements of 8q12 and 3 cases have shown rearrangements of 12q13–15. Considering the frequency of these abnormalities in benign PA, carcinomas are likely to develop at similar frequencies in both subgroups of tumors. Cytogenetic evidence of gene amplification is found in 40% of the cases (6 of 15). The true frequency of gene amplification in CexPA could in fact be higher because amplification is not always visible at the cytogenetic level. Only two of the cases with cytogenetic manifestations of gene amplification have been studied in enough detail to permit identification of the genes amplified. Interestingly, both cases have shown amplification of 12q13–15 sequences, including *HMGIC* and *MDM2* (the present case), and *CDK4* and *MDM2* .[13] The copy number of *HMGIC* is not known in the latter case. This case had also a second population of dmin with amplified *MYC* sequences. A third case had a 14q+ giant marker chromosome partially derived from 8q12-qter.[6] Whether *PLAG1* and/or *MYC* are amplified on this marker is unknown. A fourth case of CexPA had ring chromosomes of varying sizes partially derived from chromosome 2,[8] ie, a chromosome harboring the *MYCN* gene, which is known to be amplified in several tumor types including neuroblastoma. Collectively, the current as well as previous observations suggest that gene amplification and overexpression of genes in 12q14–15, including *CDK4*, *HMGIC* and *MDM2*, may be important genetic events contributing to malignant transformation of benign PA. This conclusion is supported by the following observations: amplification of *HMGIC*, *MDM2* and *CDK4* are common in certain types of malignant tumors (eg, sarcomas and malignant gliomas[44,45]); and dmin and hsr are almost never found in benign PA. Among nearly 500 cases analyzed cytogenetically only two such cases have been found.[14,46]

Whether it is *HMGIC* and/or *MDM2* that is the target gene(s) for the amplification is not known. In a study of 122 sarcomas Berner et al[44] found amplification of *HMGIC* in 13 cases and of *MDM2* in 17 cases. *HMGIC* was always coamplified with *MDM2*. The pattern of amplification in sarcomas suggested that there was preferential selection for inclusion of *HMGIC* in the amplicons. This is in line with studies showing that the HMGIC protein is abundant only in transformed cells and that there exists a correlation between overexpression of HMGIC and a malignant phenotype.[47,48] Our finding that HMGIC was highly overexpressed preferentially in the large polymorphic nuclei in the carcinomatous parts of the tumor supports these observations. Also, amplification of *MDM2* could be selected for by overexpression of the gene; the MDM2 protein is known to bind to and inactivate the TP53 tumor suppressor protein.[49]

Other recurrent abnormalities that, in addition to gene amplification, could be of importance for malignant transformation of PA are gains of extra copies of chromosome 7 and deletions of segments distal to 5q22 found in three and two cases, respectively. We and others have previously also shown that alterations of TP53 (mutation and/or overexpression) are frequent in CexPA but not in benign PA.[37] The frequency of TP53 alterations in CexPA varies in different investigations from 29 to 67%. Genetic analysis of additional cases of CexPA are necessary to determine the frequency and nature of oncogene amplification and of deletions/mutations of tumor suppressor genes as well as their significance for malignant transformation of benign PA.

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