CHARACTERIZATION OF THE MDM2 AMPLICON IN 12q14-15 AND CLINICAL CORRELATION OF MDM2 AMPLIFICATION IN CLASSICAL OSTEOSARCOMA

*Mejia-Guerrero, S; *Wunder, JS; *Gokgoz, N; *Gill, M; *Parkes, RK; +*Andrulis, IL
+*Samuel Lunenfeld Research Institute at Mount Sinai Hospital, Toronto, Canada
andrulis@mshri.on.ca

Introduction. Classical osteosarcoma (OSA) is the most common malignant tumor of the bones. It is characterized for highly aneuploid and complex karyotypes that include translocations, deletions, supernumerary chromosomes and abnormal gene copy number (amplifications). Though these aberrations must be reflected in the altered expression of a multitude of genes, it would be of clinical importance to define the ones involved in osteosarcoma pathogenesis. One of the most commonly studied genes is p53, a tumor suppressor that is frequently deleted or mutated in most cancers. Though in OSA there is no clear relationship between p53 loss and clinical outcome, it has been proposed that its increased downregulation could have a similar impact to that of the loss of the gene. This is of interest because among the most frequently amplified regions in these tumors are 12q and 17p. In the 17p region is COP3, which is part of the COP9 signalome implicated in the degradation of p53. We have previously shown that COP3 is amplified in 51% of patients with classical OSA, and is associated with a higher tumor size and a higher risk of metastasis (1).

The MDM2 gene, which is involved in ubiquitination and transport of p53 to the cytoplasm for its degradation, is located in the long arm of chromosome 12. MDM2 has been shown to be amplified in around 10% of OSA (2,3), and its overexpression can be detected in nearly 30% of the cases (4). In the present study we analyzed the frequency and clinical importance of MDM2 gene amplification. Also, to determine if there is a difference in MDM2 status between classical OSA and other tumors, we compared our data with those of parosteal sarcoma, a less aggressive form of bone cancer. Finally, since the amplicon appears to be very large, it is important to characterize the genes that could be coamplified with MDM2 in the region 12q14-15.

Methods. One hundred thirty classical OSA and 15 parosteal tumor samples were selected for analysis of MDM2 amplification. Each eligible patient provided signed consent prior to study entry as approved by each participating IREB. Amplification frequency for MDM2 and genes at 12q14-15 CPM, CPSF6, GAS41, SLC35E3, RAP1B, MDM1, IFNG and DYZK2 was evaluated by real-time quantitative PCR. Tumors with a 2-fold or greater increase in gene copy number relative to control unamplified DNA were considered to have gene amplification. Differences in clinical variables based on amplification status were statistically assessed using Fisher's exact test for categorical variables, and the exact Wilcoxon rank sum test for continuous variables. Disease-free survival was assessed using the time from diagnosis to metastasis or last follow-up if continuously free of disease. For expression analysis, RNA of each tumor was transcribed and subjected to real-time quantitative PCR.

Results. MDM2 was amplified in 16 of 130 (12.3%) classical OSA and 9 of 15 (60%) parosteal sarcomas (p=0.0001). In classical OSA the genes most frequently amplified with MDM2 were SLC35E3, carboxypeptidase-M (CPM), and CPSF6 (70-80% frequency). DYSK2, IFNG-gamma, MDM1, RAP1B and GAS41 were coamplified in around 50% of the cases. Coamplification in parosteal sarcomas was higher, with RAP1B, SLC35E3, CPM and CPSF6 as the most amplified genes (80-100% frequency), and DYSK2, IFNG, and CPSF6 as the less amplified (50-70% frequency). With the exception of CPM, there was no evidence that other genes in the 12q14-15 region analyzed were more frequently amplified than MDM2.

In classical OSA MDM2 amplification was associated with gender (31.3% amplification in male vs 68.8% in female, p = 0.023) and age at diagnosis (median, 17.8 years in non amplified vs 32.3 years in amplified, p = 0.016). There was no evidence of association with other clinical parameters (disease-free survival, systemic disease status on presentation, disease site, necrosis, tumor size, p53 mutations, or COP3 amplification status).

MDM2 mRNA overexpression was evaluated in OSA specimens. Preliminary results suggest that at least 30% of the MDM2 non-amplified tumors also overexpress the gene. Interestingly, we have also found MDM2 to be overexpressed in all seven parosteal tumors evaluated.

Discussion. MDM2 is amplified in roughly 12% of the classical OSA analyzed and in more than half of the parosteal sarcoma tumors. MDM2 expression preliminary results agree with this view: while there is an apparent 30% MDM2 overexpression in classical OSA, all analyzed parosteal sarcomas overexpress the gene. Since parosteal tumors are generally low grade and classical OSA are high grade, our results would appear to contradict the general idea that MDM2 acts as a tumor promoter. MDM2 amplification is correlated with a later development of classical OSA tumors.

However, MDM2 is not the only amplified gene in these cancers. Among the coamplified genes is IFN-gamma and CPM. It has been previously suggested that each of these genes could contribute to an enhanced immune response against tumor development (5,6). Also, in this amplicon are located, between IFN-gamma and MDM1, the genes for interleukin (IL)-22 and IL–26, which also have been related to regulation of the immune response (7).

It is, therefore, important to confirm the amplification status of IL-22 and IL-26, and to determine the expression pattern of CPM, IFN-gamma, IL-22 and IL-26. Furthermore, there are not the only genes present in the MDM2 amplicon. In other systems it has been suggested that the products of coamplified genes may act cooperatively to contribute to tumorigenesis (8). Therefore, it is possible that amplification of two or more genes at 12q14-15 in classical OSA and parosteal sarcomas may result in overexpression of proteins with synergistic and/or antagonistic effects.

References:

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