AMPLIFICATION AND OVEREXPRESSION OF COPS3 IN OSTEOSARCOMA: RELATIONSHIP TO P53 MUTATION AND PATIENT OUTCOME

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Objective. 17p11.2-p12 is frequently found to be amplified in high-grade osteosarcomas, suggesting this region may contain a candidate oncogene or oncogenes which contribute to osteosarcoma tumorigenesis. COPS3 maps to 17p11.2, and has been shown to be amplified and overexpressed in a small number of osteosarcomas. Therefore, COPS3 represents an interesting candidate oncogene involved in the development and progression of osteosarcoma.

COPS3 is a subunit of the COP9 signalosome, which has been implicated in signal transduction and ubiquitin-mediated proteolysis. COP9 signalosome-specific phosphorylation targets p53 to MDM2-mediated degradation. Amplification/overexpression of MDM2 can lead to loss of the P53 protein tumor suppressor functions in osteosarcoma, but this occurs only rarely. It has been speculated that amplification and overexpression of COPS3 in osteosarcoma may represent another route to p53 protein degradation, resulting in a phenotype equivalent to inactivation of p53 by mutation. Therefore, it has been suggested that COPS3 amplification and p53 mutation may be mutually exclusive events in osteosarcoma. In this study, we investigated the relationship between COPS3 alterations, p53 mutation and patient outcome in a large group of patients with high-grade osteosarcoma.

Methods. COPS3 gene amplification and overexpression were determined using real-time reverse transcriptase PCR. Asparagine synthetase (AS) was co-amplified with COPS3 as an internal control in each reaction. Human placenta DNA, and RNA from a pool of 11 human tumor cell lines were used as internal controls throughout the study for amplification and expression assays respectively. One hundred eighty-six high-grade osteosarcomas were evaluated in this study: 176 for DNA amplification, 129 for mRNA expression, and 117 paired samples for both DNA and RNA analysis. Tumors that exhibited a 2-fold or greater increase in gene copy number relative to control unamplified DNA from placenta were considered to be amplified, while a 2-fold increase in mRNA compared to the control cell line pool was considered evidence of overexpression. P53 gene status was determined by SSCP analysis of exons 4-10 followed by direct sequencing. Comparisons were made using Fisher's exact test or the Chi-square test.

Results. The COPS3 gene was highly amplified and overexpressed in high-grade osteosarcomas. The overall frequency of COPS3 amplification and overexpression was 37.5% (66/ 176 specimens) and 53.3% (69/ 129 specimens) respectively. P53 mutations were detected in 34 cases (34/157, 22%), and included 20 missense and 14 nonsense alterations. Surprisingly, a similar proportion of p53 wildtype osteosarcomas had COPS3 amplification (39/123 specimens; 32%) compared to tumors with p53 mutation (13/34 specimens; 38%; p=0.85). The relationship between COPS3 amplification and patient outcome was evaluated for 174 patients with available clinical outcome information. Patients with COPS3 amplification were more likely to develop metastases (38/85, 45%) than patients with a normal COPS3 gene (26/89, 29%) (p=0.04). Interestingly, it seems that patients with COPS3 amplification and p53 mutation got worse outcome compared with the patients only had either aberration, even if the p-value isn’t significant (p=0.27). 7 patients got metastasis in 12 patients with COPS3 amplification and p53 mutation (7/12, 58.3%), while 7 got metastasis in 20 patients with P53 mutation only (7/20, 35%), and 19 in 42 patients with COPS3 amplification only (19/42, 45%).

Conclusion. The finding that COPS3 is highly amplified and overexpressed in osteosarcoma supports the idea of COPS3 being a promising oncogene involved in tumorigenesis and progression. The protein product of COPS3 is thought to provide osteosarcoma cells with a growth advantage through degradation of p53 and loss of its tumor suppressor functions. In this way COPS3 may be at least partially responsible for determining the biological behaviour of osteosarcoma.

Complex genetic changes have been well characterized in osteosarcoma. We found that COPS3 amplification and p53 mutation frequently occur in the same tumors. Patients with COPS3 amplification are more likely to develop metastasis. Those patients with both p53 mutation and COPS3 amplification may be at even great risk of developing systemic disease. Therefore, COPS3 amplification and p53 mutation are not mutually exclusive, as has been previously suggested, but may be complementary events in osteosarcoma with additive biological and clinical effects. The effects of COPS3 on the pathogenesis and clinical progression of osteosarcoma require further evaluation.