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Centrosome amplification in primary sarcoma cultures and its association with malignant behavior in tumor

Valter Penna[1], Marjori Camparoto[1], Rui Reis[1], Jeremy Squire[2], Sandra Morini[1], Adhemar Longatto[1], Isabela Carvalho[1], Carla Pinheiro[1], Maicon Zanon[1]

[1)] Barretos Cancer Hospital, Brazil [2)] University of Toronto, Canada

Background - Recent studies have reported that centrosome amplification occurs in various types of malignant and borderline malignant sarcomas. In these tumours, aneuploid karyotypes are strongly associated with centrosome alterations. New evidence on centrosome clustering mechanisms has provided insights on how cancer cells survive with supernumerary centrosomes. We investigated the frequencies of centrosome clustering in primary sarcomas in association with clinical aspects.
Objective – The aim of the present study was to standardize the method for isolation and cultivation of tumor cells from samples of sarcoma tissue biopsies for centrosome analysis. and to compare findings in centrosome amplification frequency between different sarcoma subtypes.
Methods – 12 samples of sarcomas were collected from 11 patients of the Orthopedics Department at the Barretos Cancer Hospital, Barretos-SP-Brazil between January and September 2012. Primary tumor specimens were finely minced, trypsin treated and cultured in DMEM supplemented with 10% fetal bovine serum and 1% antibiotics. Cells were cultured on coverslips for 3 to 4 days, washed with phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde, and then permeabilized with Triton-X100. The UltraVision Plus detection system was used for centrosome immunostaining and analysis. The cells were incubated overnight with mouse monoclonal anti-γ-tubulin following blockade with Ultra-V-Block. After DAB exposure, the slides were subsequently stained with hematoxylin. Centrosome signals were evaluated in 100 cells to determine centrosome number frequencies.
Results – Tumors cell centrosomes were present in variable numbers, located in clusters and/or in isolated points within the nucleus. Quantitative analysis demonstrated differences in centrosome amplification frequency between the different subtypes of sarcomas. Grade I chondrosarcomas presented centrosome amplification in 48% of cells, where as Grade III or recurrent tumors presented 76% of cells with amplification and demonstrated a of 3.5-fold increase in the frequency of cluster formation. For pleomorphic, synovial and myxoid sarcoma the frequencies of clusters was the highest, ranging from 26 to 58.
Conclusion – These data related to centrosome amplification were relevant and could contribute to the understanding of the pathological diagnosis and prognosis of bone and soft tissue sarcomas.

E-mail (main author): valter_penna@terra.com.br