



# 58<sup>th</sup> National Meeting of the Italian Society of Biochemistry and Molecular Biology

Università degli Studi di Urbino Carlo Bo, Urbino  
September 14 -16, 2015



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## **WIN-induced vesiculation cooperates to the inhibition of osteosarcoma cell migration**

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**Introduction.** Animal cells release vesicles that mediate the secretion of a variety of factors in the surrounding environment affecting neighboring cells. There is increasing evidence that secreted vesicles play an important role as vehicle of intercellular communication in different biological systems and are able to influence both physiological and pathological processes. Recently, we have reported that the synthetic cannabinoid WIN55,512 is able to induce osteosarcoma MG63 cell death and negatively affect cell migration. Here, we study the effects of WIN on the induction of vesicle secretion and their possible role in WIN-dependent reduction of osteosarcoma cell migratory ability.

**Methods.** Vesicles from MG63 cells were obtained by ultracentrifuging at 140,000g media derived from cell cultures untreated and treated for 24 h with 5  $\mu$ M WIN. Purified vesicles were quantified by cytofluorimetry and by detecting acetylcholinesterase activity according to established criteria. Scratch wound healing assay was employed to monitor cell migration toward the center of a gap created in a cell monolayer. Zymographic analysis was used to evaluate metalloproteinase activities in the vesicles.

**Results.** WIN treatment induced a significant increase (about 4-fold) in the number of vesicles released by osteosarcoma cells. Wound healing assay showed that in the presence of vesicles from WIN-treated cultures, cells only partially filled the gap with respect to those conditioned with vesicles isolated from control cells which closed the gap within about 24 h. Furthermore, zymography assay showed a reduced activity of MMP-2 and MMP-9 in the vesicles obtained from WIN-treated cells.

**Conclusion.** Data indicate that the increase in the number of vesicles released after WIN treatment and/or their probable different composition can be responsible for the relevant inhibition of MG63 cell migration induced by the cannabinoid.