# Effect of $CB_2$ agonist, JWH 133, on the control of angiogenesis in a murine model of lung cancer

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Angiogenesis is one of the potential target to limit solid tumor outgrowth, including non-small-cell lung cancer (NSCLC) (Giaccone, 2007). The therapeutic efficacy of anti-angiogenic drugs has been demonstrated in clinical studies and anti-angiogenic compounds represent an important coadjuvant approach to the classic anti-cancer therapies, especially in NSCLC.

A promising class of anti-angiogenic compounds may be represented by Cannabinoids (CBs) (De Filippis and Iuvone 2009) that act through the activation of two membrane receptors, namely $CB_1$ and $CB_2$.

The aim of present study was to evaluate the involvement and role of the endocannabinoid receptors in Lewis lung carcinoma (LLC) model established in C57BL/6 mice. For the purpose, mice were injected intravenously with $2.5x10^5$ LLC cells (Sorrentino et al., 2010). Animals were treated, from $13^{th}$ to $17^{th}$ day after cell implant, with JWH 133, a $CB_2$ full agonist, at the daily dose of 0.5, 1, or 5 μg/mouse by i.p.. At various time points mice were euthanized and the left lung lobes were fixed in OCT medium; 7-μm cryosections have been cut for immunoistological analysis to measure the tumour burden. The right lung lobes were homogenized for biochemical analysis: Western blot to evaluate the expression of endocannabinoid receptors, ELISA for VEGF measurement and Proteome Profiler™ for quantification of pro-angiogenic markers.

Our results show that the expression of $CB_2$, but not $CB_1$ receptors, was significantly increased in lung of mice injected with LLC cells (13, 15 and 17 days) after cells implantation, as compared to naïve mice. On the basis of these results, we investigated the role of the $CB_2$ receptors in the lung carcinoma-dependent angiogenesis. The chronic administration of JWH133 (0.5-5 μg/mouse) significantly reduced the tumor area in LLC-implanted mice compared to PBS. Interestingly, JWH 133 (0.5-5 μg/mouse) significantly decreased VEGF expression in the lung of tumor-bearing mice compared to PBS. To confirm the anti-angiogenic effects of JWH133 we use an array kit for measuring the relative expression levels of 53 mouse angiogenesis related proteins. The array analysis revealed that the treatment with JWH133 (5 μg/mouse) reduces the activation of several pro-angiogenic mediators, compared to PBS treated mice.

In conclusion, our data demonstrate, for the first time, that the treatment with a $CB_2$ agonist, JWH 133, reduces lung tumor burden by altering the angiogenesis process in a mouse model of lung cancer.

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