



FOR HEALTH SCIENCES AND TECHNOLOGY





Development of a Macrophage-based Prognostic Scoring System and Evaluation of Bufalin as a Macrophage Phenotype Modulator in Head and Neck Cancer using 2D and 3D Models

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Tumors-associated macrophages (TAMs) are key components of the tumor microenvironment (TME) and have been shown to play important roles in the progression of head and neck cancers (HNC). Macrophages can undergo differentiation into two main functional phenotypes depending on the local tissue environment. Based macrophages on these functional phenotypes, TAMs can promote tumor progression (M2 phenotype) or inhibit it (M1 phenotype). Presence of M2 macrophages and a high ratio of M2/M1 in the TME are clinically associated with a poor prognosis for HNC patients. Analysis of TAM plasticity in HNC is largely explored and targeting M2 macrophages to repolarize them through M1 phenotype would be a promising cancer treatment strategy.

CLINICAL STUDY

Evaluation of M1 and M2 macrophage markers, respectively CD80 and CD163, in a population of 54 patients with HNC from diverse tumor locations, stages, and patient characteristics such as smoking status.



"Macroscore" is the combination of the M2/M1 and M1+M2 ratio. Patients with a high Macroscore had significantly poorer survival than those with a low score (p=0.002) meaning that patients with either a high ratio of M2/M1 and/or a high value of M1+M2 have shorter survival.

3D COCULTURE MODEL







M0-FaDu

- 14,01% CD206 - PE
- Evaluation of the expression of macrophage phenotypic markers by IF and FACS (CD86 and CD206 and the epithelial marker EPCAM) at the 7th day of coculture in different conditions.



* mRNA relative expression ($2^{-\Delta Ct}$) of M2 markers (CD206, IL10) and EMT markers (E-Cadherin ,vimentin) on dissociated FaDu and FaDu-monocyte spheroids. * = $p \le 0.05$.











M1-FaDu





 Evaluation of the expression of macrophage phenotypic markers by IF and RT-qPCR. CD206 and CD86 were labeled on M0 macrophages before and after bufalin treatment at 40nM for 48h.

CD206



Monocytes - FaDu



Antitumoral

Pro-inflammatory

cardiotonic steroid that belongs to the group of bufadienolides



Evaluation of the transcription level of M1-associated M2-associated and cytokines (IL12, IL10) in macrophage before and after bufalin treatment. T-test Exp independent (* $p \le 0.05$)



CONCLUSIONS

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Macroscore is a useful tool in categorizing HNSCC patients by macrophage infiltration and phenotype, offering valuable prognostication. • HNC cells in 3D coculture induce M2-like macrophage polarization by creating an immunosuppressive TME. Bufalin facilitates the M2 to M1 transition of macrophages in the tumor microenvironment, making it a promising immunoregulatory approach for cancer therapy.