The Exposome-Redox Interface in Oral Cancer: Mechanistic Insights into Rosmarinic Acid Action

C. Domingues[I], I. Jarak[I], A. Matos[I], F. Veiga[I], C. Vitorino[I], M. Dourado[II], A. Figueiras[I]

[I]FFUC - Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal, [II]FMUC- Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Oral squamous cell carcinoma (OSCC) incidence is projected to increase by nearly 40% by 2040, underscoring the urgent need for preventive and therapeutic strategies. Rosmarinic acid (RA), a natural polyphenol with reported anticancer properties, remains poorly explored in OSCC. Here, we investigated RA in both 2D monolayers and 3D spheroid models of OSCC to delineate its mechanistic actions at the exposome–redox interface. RA selectively reduced cell mass and metabolic activity in a dose-, time-, and cell-type-dependent manner, with the strongest effects in highly invasive OSCC cells, while sparing normal oral mucosa at therapeutic concentrations. Mechanistically, RA decreased mitochondrial membrane potential and altered redox homeostasis, as supported by metabolomic profiling that revealed glutathione depletion alongside the release of acetate and fumarate. RA further induced autophagy, as indicated by *BNIP3* and *BCNL1* upregulation and *BIRC5* downregulation, and modulated epithelial–mesenchymal transition by increasing *CADM1* while suppressing *VIM*, *CADM2*, *SNAIL1*, and *SOX9* expression. Extracellular matrix remodelling was evidenced by downregulation of *MMP-2* and *MMP-9*. Molecular docking suggested a high-affinity interaction with P-glycoprotein (−6.4 kcal/mol), while surface charge measurements in HSC-3 cells indicated partial reversal of polarity (−22.6 ± 0.3 mV vs. −26.3 ± 0.3 mV, $p < 0.0001$), consistent with impaired invasive capacity. In 3D spheroids, RA reduced both growth and metabolic activity, further supporting its potential as an anticancer agent. Importantly, its modest binding to salivary proteins highlights the feasibility of oromucosal delivery. Collectively, these findings position RA as a promising agent targeting redox pathways and cell plasticity in OSCC, warranting further investigation as monotherapy, in combination regimens, or within nanosystem-based delivery platforms.

Acknowledgments: To FCT - Fundação para a Ciência e Tecnologia, I.P., by project reference 2021.08095.BD and DOI: https://doi.org/10.54499/2021.08095.BD.