Norcantharidin anticancer activity in oral cancer cells depends on MAPK-p38-alpha signaling pathway is mediated by PP2A inhibition

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Norcantharidin, a demethylated cantharidin analog, has been shown to have significantly less toxic effects in primary rat hepatocytes and anticancer activity in our previous work. We used two unique cell lines developed by our lab representing 4-NQO-induced oral cancer cells T28 and non-tumor cells N28 obtained from tissues surrounding the induced cancer as a model to screen out whether p38 MAPK is involved in the malignant transformation processes. The results suggest an association between p38β not p38α and oral cancer development. Further experimental evidences shown that after 24h different dosages norcantharidin treatments, the IC50 of norcantharidin in T28 cells is 31μM and none scientific cytotoxicity in N28 cells. the protein level of cleaved caspase 3 was inducible through the PP2A inhibition in T28 cells within 24h. Immunoblotting of the ontogenesis MAPK isoform p38α and p38β from N28 and T28 cells, the p38β protein level was discovered higher in T28 cells than in N28 cells. After siRNA p38β treatment to the N28 and T28 cells, the apoptotic protein caspase 3 activation followed the p38β silence in T28 cell in 24h. The prediction pathway above these experiences results, p38β MAPK might inhibit the apoptosis within the oral carcinogenesis. In oral cancer therapy, p38α activates inverse the hindrance from p38β MAPK. Such as norcantharidin treatment can inhibit the PP2A and cause the p38α MAPK activation.

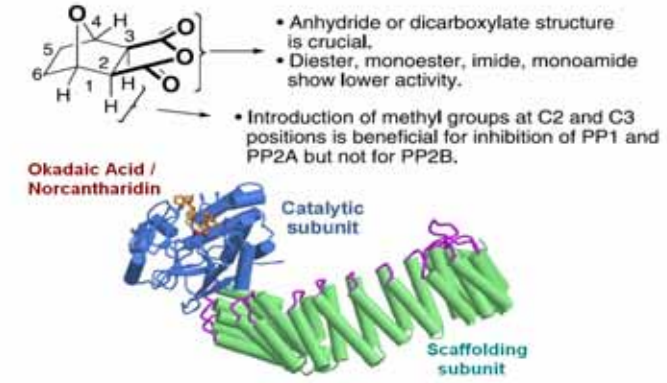

Figure 1. The structure of norcantharidin is tally with the demand for PP2A inhibitor in structure activity relationship (SAR). The predicted mode of interaction between Okadaic acid/ norcantharidin and PP2A was based on protein-protein docking.

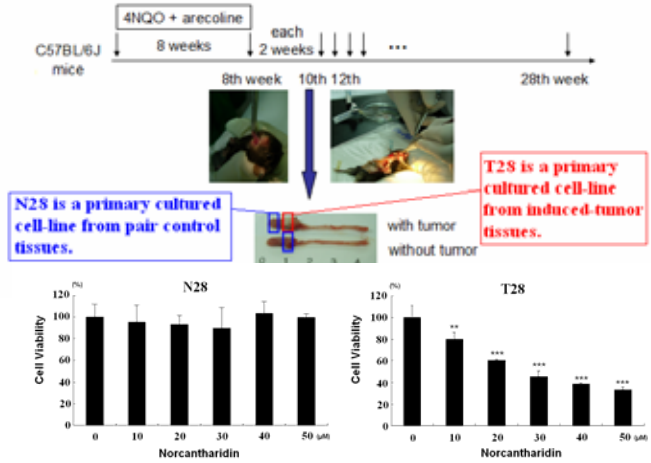

Figure 2. The oral cancer carcinogenesis was induced by 4-NQO and arecoline. N28/T28 primary culture cell-lines were isolated from pair control/ induced-tumor tissues. After 24h different dosages norcantharidin treatments, the IC50 of norcantharidin in T28 cells is 31μM and none scientific cytotoxicity in N28 cells.

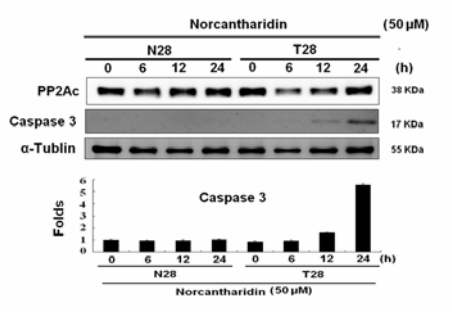

Figure 3. Immunoblotting of the PP2A and caspase 3 from the N28 and T28 cells after the norcantharidin treatments, the protein level of cleaved caspase 3 was inducible through the PP2A inhibition in T28 cells within 24h.

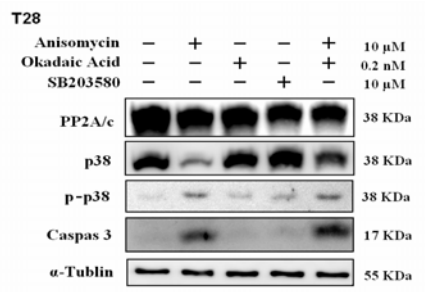

Figure 4. Immunoblotting of the PP2A, p38 and caspase 3 from the T28 cells treated with Okadaic acid (PP2A inhibitor, 0.2nM), SB203580 (p38 MAPK inhibitor, 10μM) and anisomycin (p38 MAPK activator, 10μM) for 24h. The protein level of cleaved caspase 3 was inducible through the PP2A inhibition and associated with p38 MAPK activation.

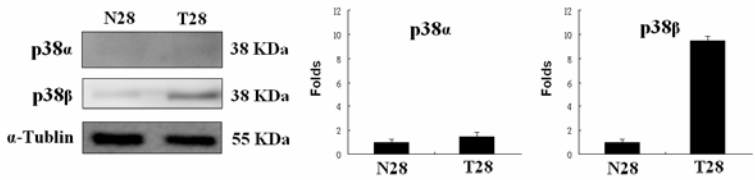

Figure 5. Immunoblotting of the ontogenesis MAPK isoform p38α and p38β from N28 and T28 cells, the p38β protein level was discovered higher in T28 cells than in N28 cells.

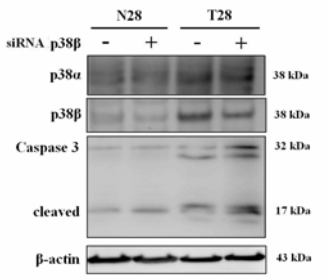

Figure 6. After siRNA p38β treatment to the N28 and T28 cells, the apoptotic protein caspase 3 activation followed the p38β silence in T28 cell in 24h.

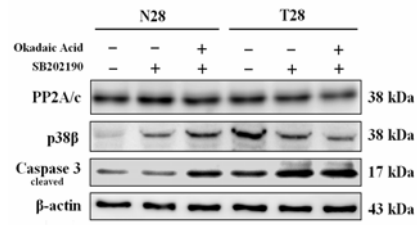

Figure 7. Immunoblotting of the PP2A, p38 and caspase 3 from the T28 cells treated with Okadaic acid (PP2A inhibitor, 0.2nM), SB202190 (p38β MAPK inhibitor, 10μM) for 24h, The protein level of cleaved caspase 3 was inducible through the PP2A inhibition and associated with p38β MAPK inhibition.

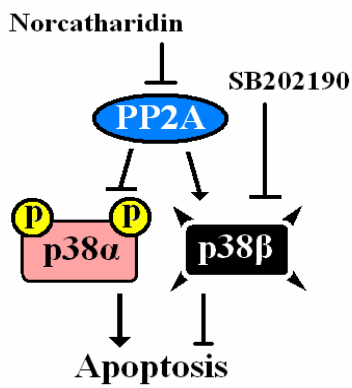

Figure 8. The prediction pathway above these experiences results, p38β MAPK might inhibit the apoptosis within the oral carcinogenesis. In oral cancer therapy, p38α activates inverse the hindrance from p38β MAPK. Such as norcantharidin treatment can inhibit the PP2A and cause the p38α MAPK activation.