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ESSENTIAL OILS FOR THE DISCOVERY OF NEW ANTHELMINTIC COMPOUNDS TESTED ON THE NEMATODE CAENORHABDITIS ELEGANS

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Parasitic nematodes of humans and animals cause diseases of major socio-economic importance globally. Control of infections in both human and veterinary medicine currently relies mainly on chemotherapy, but resistance is an increasing problem, so there is an urgent need for discovery of novel drugs. As parasitic nematodes are not ideal laboratory animals, the free-living nematode C. elegans was demonstrated to be an excellent model system for the discovery of new anthelmintics and for characterizing their mechanisms of action and resistance. Essential oils (EOs) are natural products produced by aromatic plants. EOs are complex mixtures that contain 2 or 3 major phytochemicals, which can be terpenes or aromatic compounds. We used paralysis assays of wild-type and mutant C. elegans strain to identify EOs with potential anthelmintic activities, reveal the active components, the target sites and the mechanisms of action. We found that EOs belonging to six different orders produced rapid paralysis of C. elegans and we established the half maximal effective concentration values between 0.02-1.2 percent of EOs. All EOs tested also inhibited egg hatching, a property related to anthelmintic ability. Thus, EOs mediate both rapid and long-term anthelmintic effects. We determined that trans-cinnamaldehyde (TC), a major component of C. verum EO, produces both paralysis and egg-hatching inhibition. By testing mutant worms, we identified the muscle L-AChR and GABA receptors as EOs and TC targets in vivo. Thus, by modulating two receptors with key roles in worm motility, these EOs emerge as novel sources of anthelmintic compounds. Likewise, the N-AChR mutant strain is slightly resistant to TC, thus revealing a third target receptor for terpenes. Due to the potential of EOs as sources of novel antiparasitic compounds, additional studies will be carried out to determine in more detail the molecular mechanisms of action and structure-activity relationships of their active compounds.

MAGEC2: A NOVEL MEMBER OF THE RAS/B-RAF ONCOGENIC PATHWAY TO COUNTERACT THE P53 RESPONSE IN HUMAN MELANOMA

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Ras proteins (H-, N- and K-Ras) transduce proliferation signals from growth-factor activated receptor tyrosine kinases (RTKs) through the mitogen activated protein kinase (MAPK: Raf/MEK/ERK) pathway. Activating mutations in Ras or B-Raf proto-oncogenes (i.e, RasV12 or B-RafV600E) are frequent in human melanoma. In normal cells oncogenic Ras or B-Raf activates p53 and/or pRb tumor suppressor response. We aimed to study the MAGE-I (Melanoma Antigen Genes-I) proteins involvement in wt-p53 regulation in melanoma (p53 regulators highly expressed in melanoma).

Here, we identified MageC2 protein as a p53 regulator in an oncogene-activated MAPKs context. First, we observed that MageC2 protein levels respond to serum deprivation in cultured cells. Since growth factors activate RTK/Ras, we studied Ras role, and we observed that RasV12 enhanced MageC2 levels depending on a functional MEK/ERK pathway by PD184352 MEK inhibitor treatment. MageC2 raising did not involve MageC2 expression changes and required active proteasome as indicated by MG132 treatment, and accumulated MageC2 was phosphorylated only in threonine as assessed by anti-phospho aminoacids. To study the MageC2 role in p53 regulation activity in oncogene-activated MEK/ERK condition, we generated CRISPR/CAS9 mediated MageC2 KO in A375 melanoma cells and we regulated MAPK hyperactivity with PD184352 to study p53 activity by its targets (p21, Mdm2 and PUMA) quantification, and we observed that MageC2 plays a key role as a downstream target of the B-Raf/MAPK oncogenic pathway by controlling the p53 response. Finally, gene expression analysis of TCGA skin cutaneous melanoma (n=448) through Cbioportal showed a significant inverse correlation between high MageC2 expression (RSEM>1000) and p53 targets expression (p21, BAX and PUMA) only in a Ras/B-Raf hyperactivated context (n=263). In conclusion, we propose that Ras or B-Raf downregulates wt-p53 activity by increasing MageC2 protein levels through the MAPK pathway.