Caenorhabditis elegans: a model organism to unravel the molecular targets dictating the anti-leukemic potency of EAPB02303, a member of the Imiqualines family.

Abstract

Imiqualines, analogues of the immunomodulatory drug Imiquimod, are a family of low molecular weight compounds synthesized by our team and protected by international patents. We focused on the potency of EAPB02303, lead compound of the second-generation family of Imiqualine, against Acute Myeloid Leukemia (AML), a haematological malignancy characterized by an aberrant differentiation and uncontrolled proliferation of myeloid blasts.
We demonstrated that EAPB02303 potently inhibited the proliferation of AML cells, with IC50s as low as 5 nM, thus presenting a significantly higher efficacy than the parent compound Imiquimod, or the members of the first-generation of Imiqualines. Additionally, EAPB02303 significantly downregulated the expression of proteins downstream of Ras (ERK1/2 and their phosphorylated form) 48 hours post-treatment. Of note, the Ras-MAPK signalling pathway is aberrantly activated in AML and mutations in Ras family members drive oncogenesis by increasing cellular proliferation and survival in AML.

We then used *Caenorhabditis elegans* (*C. elegans)* as a screening model system to unravel the molecular targets of EAPB02303. Indeed, *C. elegans* is extensively used as a model organism to study complex molecular processes of tumorigenesis. Mutations in signalling cascades promoting cancer in humans are conserved in *C. elegans*, and are associated with well-established phenotypes like sterility, infertility, longevity and vulva-less or multivulva formation. These phenotypic changes of the worms are reflective of aberrant deregulation of these pathways, and their implication in tumorigenesis. *let-60* gene in *C. elegans,* an ortholog of mammalian *Ras*, regulates the formation of the vulva. *Let-60* gain of function mutations lead to an abnormal proliferation of the vulval tissues in adult mutants, resulting in formation of vulval-like protrusions called "ectopic vulvas" (multivulva phenotype or "Muv"). We validated the potential targets of EAPB02303 that were revealed *in vitro* using AML cell lines in *let-60/Ras* mutant strains. We showed that EAPB02303 significantly reduced the multivulva phenotype in *let-60/Ras* mutant strains MT2124 and MT4698 (p-value < 0.001) at a dosage three times lower than a clinically used farnesyltransferase inhibitor.
In summary, we uncovered for the first time the mode of action of EAPB02303 in AML cancer cell lines *in vitro*, and we validated these targets using a simple *in vivo* model system. Our data suggests that this original compound has a Ras-inhibitory effects conferring a significant anticancer activity against human neoplasms linked with oncogenic or Ras mutations.

Keywords: Imiqualines, EAPB02303, Acute Myeloid Leukemia, MAPK signalling pathway, let 60/Ras, C. elegans