The borrelidin derivative BC194 influences the cellular response to hypoxia through modulation of threonyl-tRNA synthetase non-canonical functions

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The compound borrelidin and its derivative BC194 are potent inhibitors of threonyl-tRNA synthetase (TARS) and the process of angiogenesis. Recently, our work has connected these two functions by demonstrating that BC194 inhibits a TARS-mediated pro-angiogenic response through direct interaction with the synthetase. In addition, we suspect that TARS has a non-canonical function in hypoxia regulation sensitive to BC194 treatment. Microarray data of SKOV cells exposed to borrelidin exhibited an increase in VEGF expression. Interestingly, interactome databases indicate an interaction between TARS and the von Hippel-Lindau tumor suppressor (VHL), a master regulator of the hypoxic response. We have confirmed the ubiquitin-like, N-terminal domain of TARS as the site of this interaction using affinity precipitation techniques. While BC194 did not disrupt the TARS-VHL interaction, treatment of SKOV cells with the compound was found to reduce the stability of hypoxia inducible factor alpha (HIFα), the down-stream target of VHL. To further investigate the mechanism of BC194 we used mass spectrometry on affinity precipitated TARS lysates to identify novel interactions related to the hypoxia system. We focused on eukaryotic elongation factor 1 alpha (eEF1α) as reports indicate non-canonical functions in VHL nuclear-cytoplasmic translocation. The TARS-eEF1α interaction was confirmed via affinity precipitation but was not disrupted by BC194. Subsequently, we investigated the effects of TARS on eEF1α GTP-cycling, a process important for normal functions of the enzyme. Remarkably, we identified a novel GTPase activity of TARS itself sensitive to the availability of its canonical substrates: ATP, threonine, and tRNA^Thr. Together these data implicate a role for TARS in nutrient sensing that mediates the interconversion between canonical and non-canonical functions of the enzyme by GTP cycling.