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MEETING ABSTRACT

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The ferroptosis and MEK1/2 inhibitor U0126 improves functional outcome after intracerebral hemorrhage in mice independent of ERK1/2

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Background: Intracerebral hemorrhage (ICH) is a devastating neurological disease without effective treatment options. Neuronal cell death induced by the blood breakdown products hemoglobin and hemin after ICH is executed by regulated cell death mechanisms including ferroptosis, necroptosis, and autophagy. Ferroptosis is a caspase-independent form of regulated necrosis that is activated by oxidative stress induced by glutathione depletion, enzymatic derived reactive lipid species, and hemin. Recently, we identified the MAP kinase kinase 1/2 (MEK1/2) inhibitor U0126 as an anti-ferroptotic agent that abrogated neuronal cell death in experimental models of ICH *in vitro*. In this study, we evaluated hyperactivation of extracellular signaling kinase 1/2 (phospho-ERK1/2) as a target for ICH therapy.

Methods: We performed careful dose finding of U0126 and examined its effect on functional recovery after collagenase-induced ICH in mice. Further, we verified the molecular knockdown of phospho-ERK1/2 by overexpressing MAP kinase phosphatase (MKP) for its ability to protect neurons from ferroptosis after hemorrhagic stroke in addition to using chemically diverse inhibitors of MEK. In search for the mechanism of U0126, we performed an unbiased phosphor-proteome analysis.

Results: We demonstrate that the ferroptosis and MEK1/2 inhibitor U0126 improves functional recovery after ICH in mice. Whereas ICH leads to chronic hyperactivation of ERK1/2, U0126 prevented hemin-induced ferroptosis independent of its ability to inhibit ERK1/2 signaling. In contrast to classical ferroptosis in neurons or cancer cells, chemically diverse inhibitors of MEK did not block hemin-induced ferroptosis, nor did the forced expression of the ERK-selective MAP kinase phosphatase 3. We further show that phospho-ERK1/2 accumulates in the cytoplasm and is therefore unable to induce MKP1 and MKP3, its negative regulators, leading to chronic hyperactivation. Remarkably, our unbiased phosphoproteome analysis revealed dramatic differences in phosphorylation induced by classical *vs.* ICH-induced ferroptosis and provides novel insights into the mechanism of U0126.

Discussion: Taken together, our data suggests that the anti-ferroptotic agent U0126 promotes functional recovery independent of its ability to abrogate the chronic hyperactivation of ERK1/2 after ICH. These studies define distinct subtypes of neuronal ferroptosis and provide a template on which to build a search for U0126's effects in a variant of neuronal ferroptosis.

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Keywords: brain hemorrhage – cell death – ERK – ferroptosis – MAP kinase – stroke

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