

HES1 is a Novel Monoubiquitinated Partner of the FA Core Complex

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Objectives: FA is characterized by bone marrow failure, developmental defects and progressive hematopoietic stem cell (HSC) loss. The Notch1 pathway is implicated in development and in expansion as well as maintenance of HSC, via its effector HES1. We have already shown that HES1 interacts with the FA core complex, linking both FA and Notch1 pathways. We sought to determine the functional role of this novel HES1-FA complex interaction and its implication in FA.

Methods: To determine the functional significance of HES1 interaction with the FA core complex, HES1 immunocomplexes were purified and analyzed by mass spectrometry. Specific siRNA against HES1 and Notch1/HES1 inhibitor were used to knockdown HES1 expression. The FA complex function/formation was tested in cells with knockdown HES1 using MMC cell viability, FANCD2 monoubiquitination and foci formation. Ubiquitination of HES1 was measured by immunoprecipitations with epitope-tagged wildtype or K48R ubiquitin, which prevents the formation of poly-ubiquitin chains. HES1 monoubiquitination was confirmed by endogenous ubiquitin pulldowns in normal and FA mutant cells

Results: FA core complex proteins are found in HES1 immunocomplexes. Notch1/HES1 inhibitor abrogates interactions between FA core complex proteins, indicating that HES1 is required for the FA core complex stability and formation. HES1 downregulation using specific siRNA results in cellular hypersensitivity to MMC, a hallmark of FA cells. Moreover, HES1 downregulation is also corresponding to reduced FANCD2 monoubiquitination and foci formation in response to DNA damage. In addition, HES1 is monoubiquitinated, which is dependent on a functional FA complex/pathway.

Conclusions: The FA core complex partner, HES1, is required for a functional FA complex/pathway. HES1 is a novel substrate of the FA core complex E3 ligase activity.

Translational Applicability: The hematopoietic stem cell loss characteristic of FA is poorly understood. Functional characterization of the FA/HES1 interaction is a crucial step for the development of novel strategies aimed at HSC expansion as a means to correct the bone marrow failure FA patients.