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CORRESPONDENCE

Chronic myeloproliferative neoplasms

The S505A thrombopoietin receptor mutation in childhood hereditary thrombocytosis and essential thrombocythemia is S505N: single letter amino acid code matters

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Dear Editor,

Myeloproliferative neoplasms (MPNs) are rare diseases in children [1] when compared to adults [2]. Targeted sequencing revealed differences in the mutational landscape of pediatric and adult MPNs [1]. Children exhibit a lower frequency of mutations in the MPN driver genes *JAK2*, *MPL*, and *CALR*, and a much higher proportion of children exhibit no mutation among the 104 classically involved genes; these children exhibiting a trend towards essential thrombocythemia (ET) [1]. We were surprised to read about an activating thrombopoietin receptor (TpoR/Mpl) S505A transmembrane domain (TM) mutation in childhood hereditary thrombocytosis (HT) and ET [3–5].

In adults, sporadic and hereditary thrombocytosis cases have been linked to a different mutation at the same position, namely Mpl S505N [2, 6]. Substituting Ser505 with Asn induces hydrogen bonding between the Asn residues and dimerization of Mpl TM leading to signaling in the absence of ligand [7, 8]. A very different conformation and packing would occur in the S505A mutant [8]. More recently, a poster at the 23rd European Hematology Association Meeting in June 2018 reported that among 23 children with ET, 87% harbored the Mpl S505A mutation, while 13% carried the Mpl V501A mutation [5].

We constructed the cDNA for the Mpl S505N, S505A, V501A, a combination of V501A/S505A and assessed the ability of these mutant proteins to induce basal and ligand-dependent activation of the signal transducer and activator of transcription STAT5 in standard STAT5 luciferase dual reporter assay [8, 9]. This assay can easily discriminate between constitutively active and physiologic Mpl receptors [8, 9]. As depicted in Fig. 1, while Mpl S505N was constitutively active, S505A was not, although it responded to ligand. Interestingly, V501A, which is placed one turn of the helix upstream of S505 induces constitutive activation. We interpret the latter result as dimerization promoted by close packing of helices at a crossing point around Ala residue at 501 [8]. Interestingly, the ligand-independent activity of V501A was not enhanced by the simultaneous presence of S505A in the double V501A/S505A mutant (Fig. 1). We asked whether activation depended on the small volume of Ala at 501 allowing tight crossing of helices, or if it involved a specific interface. Mutating V501 to the smallest residue Gly (V501G) did not result in ligand-independent activation (Fig. 1), suggesting V501A involved a more specific dimeric conformation.

The original study reporting Mpl mutations in HT clearly described the Mpl S505N (Ser505Asn) activating mutation, as shown by the nucleotide sequence [10]. Careful examination of the studies citing the Mpl S505A mutation in HT and ET [3–5] led us to discover that the single letter code for asparagine used was A and not N. This led to the widespread citation of Mpl S505A instead of Mpl S505N in HT and ET. The Mpl V501A mutation found in pediatric patients [5] is shown here to be constitutively active and likely relevant for the disease.

In conclusion, the HT and ET Mpl S505A mutation is in fact S505N, and therefore this should be corrected in the

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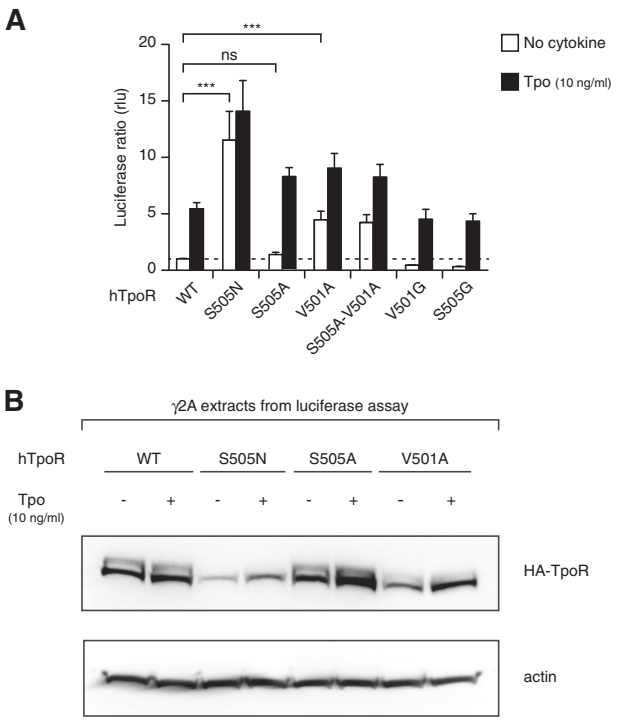

Fig. 1 Lack of ligand-independent STAT5 transcriptional activity for Mpl S505A. γ2A cells were transiently transfected with the indicated HA-Mpl variants, along with cDNAs coding for JAK2, STAT5 and dual luciferase reporters reflecting STAT5 transcriptional activity [8, 9]. Shown are averages of four-independent experiments, each performed in triplicate + SEM. ***$p < 0.001$, ns non-significant, non-parametric multiple comparison Steele test with control. Western blot analysis with anti-HA antibodies confirms HA-TpoR total protein levels

various reviews and primary research articles, as was correctly reported in the 2007 study of Teofili et al. [10].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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