ELSEVIER

Blood 142 (2023) 3817–3818

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

101.RED CELLS AND ERYTHROPOIESIS, EXCLUDING IRON

NXPE2 Is the Target of Ter-119 When Complexed with Gypa in Mice

Gregory R Keele, PhD[1], Nadia Holness[2], Arijita Jash, PhD[3], Ariel M Hay[3], Sarah Ewald, PhD[2], Gary A Churchill, PhD[4], Angelo D'Alessandro, PhD[5,6], Monika Dzieciatkowska, PhD[7,8], James C Zimring, MDPhD[9]

[1] RTI International, Research Triangle Park, NC
[2] University of Virginia School of Medicine, Charlottesville
[3] University of Virginia, Charlottesville, VA
[4] Jackson Laboratory, Chapel Hill, NC
[5] Biochemistry and Molecular Genetics, University of Colorado Anschutz Medical Campus, Aurora, CO
[6] Department of Biochemistry and Molecular Genetics, University of Colorado Anschutz Medical Campus, Aurora, CO
[7] University of Colorado, Aurora, CO
[8] University of Colorado Denver - Anschutz Medical Campus, Aurora, CO
[9] University of Virginia School of Medicine, Charlottesville, VA

Background: Red blood cells (RBCs) have a complex biology that allows regulation of metabolism, cellular rigidity, ion exchange, pH, and molecular communication with the vasculature. A central regulator of these biologies are macrocomplexes of integral membrane proteins that are both linked to cytoskeletal proteins and also differentially bind to metabolic enzymes based upon oxygenation status of the RBC. RBC surface staining, co-immunoprecipitation, and cross-linking proteomic approaches have generated a model of the macrocomplex encompassing Band 3, Rh, RhAG, CD47, glycophorin A, glycophorin B, and LW. TER-119 is a monoclonal antibody with exquisite specificity for the erythroid lineage in mice. However, the molecular target of TER-119 has remained unknown. TER-119 immunoprecipitates 4 specific protein bands, two of which are glycophorin A (GYPA) monomers or homodimers respectively; however, TER-119 is non-reactive to GYPA when assayed through Western blots and flow cytometry with erythroid cell lines expressing GYPA. These data led the originators of TER-119 to conclude it binds to a GYPA associated protein, but not GYPA itself. In contrast, others have concluded that TER-119 binds GYPA directly, and have inferred GYPA biology based upon TER-119 reactivity.
Methods: TER-119 reactivity with peripheral blood RBCs from mice or from transfected HEK cells was determined by flow cytometry. Mice were genotyped using a dense array of 143,000 markers. Quantitative trait loci (QTL) analysis was carried out using TER-119 staining as a trait. cDNA for the open reading frames of the variants of interest in candidate genes or GYPA were cloned into eukaryotic expression vectors drive by the CMV promoter. HEK cells were transfected with expression vectors, in combination with a GFP expression plasmid. Cells were stained with TER-119 48 hours post transfection and analyzed by flow cytometry, gating on GFP positive cells to isolate the transfected population. Hematopoietic lineage expression of mRNA was obtained from the BloodSpot database.
Results: The Diversity Outbred mice (DO mice) are an outbred population descended from 8 parental strains (A/J, C57BL/6J, 129S1/SvlmJ, NOD/ShiLtJ, NZO/HILtJ, CAST/EiJ, PWK/PhJ, and WSB/EiJ). While analyzing RBC biology phenotypes in DO mice, using TER-119 as a marker of RBCs, we serendipitously observed TER-119 non-reactivity in peripheral blood of 5 out of 550 DO mice. QTL analysis using TER-119 reactivity as a trait identified a highly significant QTL (genome-wide adjusted p-value <1.11e-16) driven by alleles specific to WSB/EiJ mice. Analysis of each of the parental strains confirmed that only peripheral blood from WSB/EiJ mice was non-reactive with TER-119. The QTL region contained 4 genes with missense mutations specific to the WSB/EiJ strain (HTR3a, HTR3b, NXPE2, and NXPE4). TER-119 was weakly reactive with HEK cells transfected with NXPE2 (but not the other genes). However, TER-119 became strongly reactive with HEK cells co-transfected with NXPE2 and GYPA (but not the other genes co-transfected with GYPA). There is no GYPA coding variation present in 8 DO parental strains. NXPE2 mRNA is highly expressed in the murine erythroid lineage with lower expression in B cells and granulocytes.
Discussion: These data demonstrate that TER-119 reactivity is conferred by co-expression of NXPE2 and GYPA. NXPE2 is a single pass transmembrane protein and the weak reactivity of TER-119 with NXPE2 alone suggests that TER-119 recognizes an epitope on NXPE2 that is allosterically enhanced by complexing with GYPA. However, other binding scenarios cannot be ruled out. These data are important for three reasons. First, they identify NXPE2 as a hitherto unrecognized surface protein specific

to the erythroid lineage in mice. Second, they raise the possibility that the models of the macrocomplex are incomplete. Third, these findings may allow re-interpretation of existing data that assumed TER-119 recognized GYPA. Additional studies are required to determine what function (if any) NXPE2 plays in erythropoiesis in mice and whether this function translates to human NXPE2 or a homologous gene.

Disclosures D'Alessandro: *Omix Technologies Inc:* Current equity holder in private company; *Macopharma:* Consultancy; *Hemanext Inc:* Consultancy. **Zimring:** *Rubius Therapeutics:* Consultancy.

https://doi.org/10.1182/blood-2023-178404