NIH Public Access
Author Manuscript
Hum Antibodies. Author manuscript; available in PMC 2014 July 07.

Published in final edited form as:
Hum Antibodies. 2013 ; 22(0): 21–29. doi:10.3233/HAB-130268.

Developing and mature human granulocytes express ELP 6 in the cytoplasm

Lori A. Wagner[a,*], **Shuping Wang**[b], **Elizabeth A. Wayner**[c], **Clarissa Christensen**[d], **Sherrie J. Perkins**[b], **Gavin W. Ward**[a], **Robert B. Weiss**[e], **Diane M. Dunn**[e], **Michael J. Redd**[f], **Gerald J. Spangrude**[g], and **Gerald J. Gleich**[a]

[a]Department of Dermatology, University of Utah, Salt Lake City, UT, USA

[b]Department of Biochemistry, University of Utah, Salt Lake City, UT, USA

[c]Ben and Catherine Ivy Center for Advanced Brain Tumor Treatment, Seattle, WA, USA

[d]BioFire Diagnostics Inc., Salt Lake City, UT, USA

[e]Eccles Institute of Human Genetics, University of Utah, Salt Lake City, UT, USA

[f]2000 Circle of Hope Huntsman Cancer Center, Salt Lake City, UT, USA

[g]Department of Hematology, University of Utah, Salt Lake City, UT, USA

Abstract

BACKGROUND—c3orf75 is a conserved open reading frame within the human genome and has recently been identified as the Elongator subunit, ELP6 [1]. The Elongator enzyme complex has diverse roles, including translational control, neuronal development, cell migration and tumorigenicity [2].

OBJECTIVE—To identify genes expressed early in human eosinophil development.

METHODS—Eosinophilopoiesis was investigated by gene profiling of IL-5 stimulated $CD34^+$ cells; ELP6 mRNA is upregulated. A monoclonal antibody was raised to the recombinant protein predicted by the open reading frame.

RESULTS—ELP6 transcripts are upregulated in a human tissue culture model of eosinophil development during gene profiling experiments. Transcripts are expressed in most tissue types, as shown by reverse-transcriptase PCR. Western blot experiments show that human ELP6 is a 30 kDa protein expressed in the bone marrow, as well as in many other tissues. Flow cytometry experiments of human bone marrow mononuclear cells show that ELP6 is expressed intracellularly, in developing and mature human neutrophils, eosinophils and monocytes.

CONCLUSIONS—ELP6 is expressed intracellularly in developing and mature granulocytes and monocytes but not in lymphocytes and erythrocytes.

© 2013 – IOS Press and the authors. All rights reserved

*Corresponding author: Department of Dermatology, University of Utah, 30 North, 1900 East, Salt Lake City, UT 84132, USA. Tel.: +1 801 230 7290; wags47@gmail.com. .

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Keywords

Granulopoiesis; ELP6; bone marrow; antibody; human

1. Introduction

c3orf75 (TMEM103, ATP1, Q9NXJ3, AK000218, FLJ20211 and most recently, ELP6) is a conserved open reading frame within human chromosome 3 (Gen Bank: CH471055.1, NM_001031703.2,CM000254.1). c3ORF75 was recently reported to encode ELP6, a subunit of the Elongator complex, and to have a function in cell migration and tumorigenicity [1]. Elongator was initially described as an RNA polymerase II associated transcription factor, but has since been reported to have functions as diverse as α-tubulin and bruchpilot acetylation, paternal genome demethylation, and translational control via tRNA modification [1–9]. Elongator is a 900 kDa holoenzyme consisting of two copies each of the six ELP subunits. The crystal structure of ELP4-6 subcomplex shows that the subunits are arranged in a tetrameric fashion [9]. ELP4-6 has a RecA-ATPase like fold in each subunit and is able to bind tRNA and hydrolyze ATP [9–11]. Elongator is required for the addition of 5-methoxycarbamoylmethyl and 5-carbamoyl methyl groups on wobble position uridine in tRNA; this may alter codon usage, affecting translational control [5]. Elongator may also interact with nascent mRNA emanating from RNAPII and thus have a role in transcript elongation and transcriptional control [12].

Mutations in the Elongator subunit, ELP1, cause familial dysautonomia, a severe neurological defect in humans, and also cause neuronal defects in C. elegans and drosophila [13,14]. Neuronal defects may stem from Elongator's role in tubulin acetylation and cell migration or from alterations in translational control [9,15]. Yeast elp deletion mutants grow slowly, whereas mice elp1 knockouts are embryonic lethal [13]. A deletion of any ELP1-6 subunit prevents yeast ochre suppressor tRNA from reading stop co-dons, supporting Elongator's role in tRNA modification [16]. Human ELP6 was reported to have a function in cell migration and tumorigenicity [1]. In drosophila, *elp3* mutants affect larval development and reduce the number of hemocytes, which are analogous to human myeloid lineage cells [17] Our data using a mouse monoclonal antibody to human ELP6 shows protein expression in many tissues, including developing myeloid cells in bone marrow as well as myeloid cells in peripheral blood. Our data also supports the recent identification of human c3orf75 as ELP6 based on protein similarity, and shows expression is limited to the myeloid lineage in hematopoietic cells.

2. Materials and methods

2.1. CD34$^+$ progenitor isolation and culture

CD34$^+$ cells were isolated from umbilical cord blood mononuclear cells (UCMC) using the Miltenyi Midi Macs System and Direct CD34$^+$ Progenitor Isolation Kit or StemSep CD34$^+$ Human Selection kit. Cells were cultured in RPMI with glutamine, penicillin/streptomycin, 10% Fetal Calf Serum (Hyclone) and 5 ng/ml IL-5 (R&D Systems) or 2 U/ml epoietin-α [18]. Collection of de-identified UCMC was approved by the University of Utah's

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Institutional Review Board. Bone marrow mononuclear cells (BMMC) were purchased from AllCells (Emeryville, CA).

2.2. Microarray

CD34$^+$ cells were isolated from human umbilical cord blood. Following 24 hours of culture in 5 ng/ml IL-5 or 2 U/ml epoietin-α, total RNA was isolated using the RNeasy mini kit (Qiagen, Valencia, CA) and amplified using the Arcturus Riboamp RNA amplification kit. Labeling and hybridization of amplified anti-sense RNA to U133A and B DNA chips was done in triplicate according to the manufacturer's (Affymetrix, Santa Clara, CA) instructions. Data were analyzed by Gene Chip-Robust Multiarray Averaging (GC-RMA), and rank sum analysis was used to identify genes that are transcriptionally up-regulated relative to control [18].

2.3. Quantitative RT-PCR

First strand human tissue cDNA normalized to five housekeeping genes was purchased (Clontech, Mountain View, CA) or isolated from sorted BMMC using RNeasy micro kit (Qiagen, Valencia, CA). PCR conditions were 0.2 mM dNTPs, 0.5 uM primers, 1/2000 Sybr Green I, 0.5 U Amplitaq (Applied Biosystems, Carlsbad, CA), 0.1ug Taqstart antibody (Clontech, Mountain View, CA), 4mM PCR buffer (Idaho Technology, Salt Lake City,UT) and 4ul 10X diluted cDNA in a 20 ul reaction. PCR was done in triplicate on a LightCycler2 (Roche Applied Science, Indianapolis, IN), and averages and standard error of the second derivative max (LightCycler Data Analysis v3.5) were plotted. Sorted cells were normalized to GAPDH. Parameters were 94°C for 0 seconds, 60°C for 20 seconds, 45 cycles. Primers for ELP6 are TMEM103F and R, 5′ GTACCCGGTGCTGTTG AND 5′CCCGCAGTATCAGATGG, respectively. Primers for GAPDH are: GAPDHF 5′ TCTCTGCTCCTCCTGTT, GAPDHR 5′CAAGCTTCCCGT TCTCA.

2.3.1. Recombinant ELP6—ELP 6 from cDNA clone, AK000218 (http://www.ncbi.nlm.nih.gov/nuccore/AK000218), was cloned into pDest17 (Invitrogen, Grand Island, NY) and transformed into Rosetta cells (Novagen, Rockland, MA). Cells were induced using IPTG and recombinant ELP6 was purified using Probond Resin (Invitrogen, Grand Island, NY) under denaturing conditions, and analyzed by SDS-PAGE for purity.

2.3.2. Monoclonal antibody—The monoclonal antibody to human ELP6 (P5A7-1) was developed by raising antibodies to a recombinant ELP6 protein (1.5 mg.ml) purified from E. coli and injected into mice [19].

2.3.3. Western blot—Human tissue lysates were purchased (Biochain, Newark, CA). Western blots were done with 1:100 P5A7-1 hybridoma supernatant as a primary antibody and donkey-anti-mouse infrared tagged (800 nm) secondary antibody according to manufacturers protocol, with the exception of omission of 0.1% SDS substituting for the Tween 20 during the secondary antibody binding (Licor, Lincoln, NE). Anti-human GAPDH was used sequentially as a primary antibody at a concentration of 1:1000 (Qiagen, Valencia, CA). Westerns were digitized using an Odyssey (Licor, Lincoln, NE).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

2.3.4. Flow cytometry—IgG was purified from P5A7-1 hybridoma supernatant using a Protein A/G column (ThermoFisher Scientific, Rockford, IL). 135 ul of 0.75 mg/ml P5A7-1 was labeled with 27 ul fluorescein (4 mg/ml) in a $NaHCO_3/Na_2CO_3$ buffer pH 9.2 for 30 minutes at room temperature and purified on a buffer exchange column. 2.5 $X10^5$ cells were labeled with APC labeled antibodies and eFluor 450 (eBioscience, San Diego, CA) for 1 hour on ice in PBS/10% FCS. Cells were washed with PBS, fixed in 100 ul 10% formalin for 20 minutes at room temperature, and washed with permeabilization buffer (0.1% saponin). P5A7-FITC was added at 3.8 ug/ml final concentration in permeabilization buffer for 20 minutes at room temperature in the dark. Following washes and resuspension in PBS, cells were analyzed on a FACS Canto II or sorted on an Aria Cell Sorter to at least 90% purity, cytospun onto slides, stained with Wright Giemsa and 600 cells were enumerated and identified by light micoscopy.

2.3.5. Immunofluorescent microscopy—BMMC were labeled for flow cytometry with P5A7-1 FITC or mIgG-FITC, as per flow cytometry protocol, cytospun onto slides, and cover slips were applied with Prolong Gold antifade reagent with DAPI (Molecular Probes, Life Technologies). Slides were photographed on a FV 1000 XY inverted confocal Olympus IX81.

3. Results

Our investigation of the c3orf75 gene began with the identification of transcripts that are increased in a model of early human eosinophil development. The model system utilized $CD34^+$ cells from umbilical cord blood stimulated with interleukin-5 (IL-5) for 24 hours *in vitro* to initiate eosinophilopoiesis. $CD34^+$ cord blood cells stimulated by epoietin-α for 24 hours were used as a control for general cell proliferation due to cytokine stimulation. Gene expression profiling of early eosinophil development on the U133-A chip showed increased transcript levels of pro-inflammatory cytokines, chemokines, transcription factors and a novel gene, *EGOT* (Eosinophil Granule Ontogeny Transcript) [20]. The unpublished data from the U133-B chip showed upregulation of several possible non-coding RNAs in the 3′ untranslated regions of known genes, and a hypothetical protein, which recently has been identified as ELP6 (Supplemental data, Table 1). We initially chose C3orf75/ELP6, for further study because, at that time, it was a conserved, hypothetical protein of unknown function.

C3orf75/ELP6 has an open reading frame (ORF) of 266 amino acids (Affymetrix identifier 229864_at, Genbank NCBI Reference Sequence: NM_001031703. 2) and is located on human chr3p21.31. Several splice variants of ELP6 ranging from 7-10 exons are represented by cDNA sequences in the Genbank database. ELP6 has homologues in vertebrates such as mouse, rat, dog, opossum, chimpanzee, chicken and zebrafish, as well as arabidopsis and yeast. ELP6 has 53% amino acid identity with the 264 amino acid zebrafish homolog on chromosome 16 and 84% identity with the mouse gene on chromosome 9.

Gene expression of ELP6 at the mRNA level was investigated by real-time quantitative reverse transcriptase PCR (RT-PCR) of pooled cDNA from multiple donors, normalized to 5 housekeeping genes. The ELP6 transcript is ubiquitously expressed but is lowest in brain

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

and peripheral blood and highest in prostate, testis, fetal liver, pancreas, spleen and thymus (Fig. 1A). BMMC flow sorted into lymphocytes, monocytes and granulocytes showed that the granulocyte subset has higher levels of ELP6 mRNA than flow sorted lymphocytes or monocytes (Fig. 1B).

Protein expression was investigated by raising a monoclonal antibody, P5A7-1, to recombinant human ELP6 protein. Mice were injected with purified human recombinant histidine tagged ELP6 grown in *E. coli* and monoclonal antibodies were tested for reactivity with the recombinant human protein by western blot (Fig. 2A). Note that recombinant protein is larger than native protein due to the addition of a histidine tag and cloning sites. Also, breakdown products of ELP6 are abundant in recombinant ELP6 protein purified from *E. coli*. The full length purified and recombinant breakdown products in Fig. 2A have been identified as c3orf75/ELP6 by mass spectrometry (L. Wagner, unpublished data). P5A7-1 was used to probe western blots of reduced human protein lysates (Biochain, Hayward, CA); a single band corresponds to a protein of approximately 30 kDa, as expected from the DNA sequence and also corroborating the recent data showing that c3orf75 is the Elongator subunit, ELP6. ELP6 protein is extremely labile and lysates cannot be freeze-thawed without degradation (L. Wagner, unpublished data). ELP6 is expressed strongly in bone marrow and absent in brain, uterus and testis, although this absence may be due to degradation of this unstable protein or low levels of the protein.

The original gene expression profiling showed that ELP6 was upregulated during eosinophil development and ELP6 is present in BMMC; therefore, we investigated the gene expression of ELP6 in BMMC subsets by flow cytometry (Fig. 3, Table 1). ELP6 is expressed on approximately 10–50% of permeabilized bone marrow mononuclear cells, depending on the person; expression is intracellular, as no ELP6 is detected with extracellular staining (Wagner, unpublished data). There are bright and dim subsets of ELP6, shown most strikingly by CD33 (a myeloid developmental marker) staining ($CD33^{bright}$ $ELP6^{dim}$ and $CD33^{dim}$ $ELP6^{bright}$ subsets). Furthermore, subsets of $CD14^{+}$ cells (monocytes, $ELP6^{dim}$), $CD15^{dim}$ (granulocytes, $ELP6^{bright}$), $CD16^{+}$ cells (neutrophils, $ELP6^{dim}$) and $IL5R^{+}$ or $CCR3^{+}$ cells (eosinophils, a mix of $ELP6^{bright}$ ELP6–) express ELP6; therefore, the gene is expressed during granulocyte and monocyte development. ELP6 is not expressed in lymphoid, basophils and erythroid cells and only dimly in some $CD34^{+}$ hematopoietic progenitor cells (Table 1), suggesting that ELP6 is expressed in cells developing from the common myeloid progenitor, (CMP). Intermediate CD45 expression versus high side scatter shows that $ELP6^{+}$ cells are granulocytic [21].

To further identify ELP6 expression in BMMC subsets, $ELP6^{-}$, $ELP6^{dim}$ and $ELP6^{bright}$ BMMC cells were segregated by fluorescent activated cell sorting, in duplicate from separate bone marrow donors, cytospun onto slides, stained and identified. $ELP6^{-}$ cells were greater than 90% erythroid lineage and lymphocytes. $ELP6^{dim}$ and ELP6 ^{bright} slides were mainly myelocytes and metamyelocytes (Table 2), the precursors to neutrophils, eosinophils and monocytes. Some monocytes are present in $ELP6^{dim}$ fractions in BMMC. The presence of small numbers of lymphocytes or erythrocytic precursors in $ELP6^{dim}$ and ELP6 ^{bright} fractions are likely due to contamination, as purities greater than 90% are difficult to achieve by cell sorting.

NIH-PA Author Manuscript

The location of ELP6 in BMMC cells was investigated by staining with P5A7-FITC (green) or mouse IgG-FITC (green), as a negative control and DAPI (blue), a nuclear stain, and photographed on a FV-1000-XY inverted confocal microscope (Fig. 4). Staining for ELP6 was in the cytoplasm. No nuclear staining was observed.

Flow cytometry of mature leukocytes from buffy coats showed similar results to developing bone marrow cells, with the majority of neutrophils ($CD16^{+}$) and approximately half of eosinophils ($IL5R^{+}$ and $CCR3^{+}$) Fig. 5, Table 3) expressing $ELP6^{bright}$. Basophils also express $IL5R^{+}$ and $CCR3^{+}$; however the high affinity IgE receptor, which is unique to basophils, was not co-expressed with ELP6 (Table 3). ELP6 is expressed brightly in $CD13^{+}$ and $CD15^{+}$ cells (granulocytes); however, ELP6 is expressed only dimly in $CD14^{bright}$ monocyte subsets. As in bone marrow, ELP6 was not expressed in lymphocytes. ELP6 was also not expressed in contaminating red blood cells (data not shown). Therefore, ELP6 is expressed in granulocytes in both BMMC and mature leukocytes; however ELP6 is expressed dimly in mature monocytes.

4. Discussion

Myeloid cells including neutrophils, eosinophils and monocytes develop in the bone marrow. Neutrophils are the most abundant white blood cell in humans and play a critical role in protection from infections. Eosinophils constitute 0.5–2% of blood and are associated with many allergic diseases including asthma, atopic dermatitis, drug reactions and chronic urticaria [22]. Monocytes are circulating blood leukocytes that play important roles in the inflammatory response, are essential for the innate response to pathogens, and are also involved in the pathogenesis of inflammatory diseases, including athe- rosclerosis. These three cell types are derived from bone marrow hematopoietic stem cells that develop into a common myeloid progenitor (CMP) [23]. Some evidence suggests that the eosinophil lineage branches off as an $IL\text{-}5R^{+}$ subset of the previously identified CMP and becomes committed prior to or during the CMP stage [24]. The IL5R− CMP develops into the granulocyte macrophage progenitor (GMP), which matures into neutrophils and monocytes. We show that ELP6 is expressed in the cytoplasm of developing and mature eosinophils and neutrophils. ELP6 is also dimly expressed in peripheral blood monocytes, and during monocyte development in the bone marrow.

CD16 expression increases during neutrophil development and is expressed strongly on mature eosinophils. ELP6 is expressed in $CD16^{+}$ cells in peripheral blood, showing that ELP6 is expressed on mature neutrophils; however, most $CD16^{+}$ cells in the bone marrow don't express ELP6 strongly. CD14 is expressed during granulocyte development and on developing and mature monocytes. ELP6 is expressed dimly in $CD14^{+}$ BMMC and in peripheral blood. The IL5R is expressed on committed eosinophil progenitors and mature eosinophils as well as basophils. IL5R is co-expressed with ELP6 in about one third of $IL5R^{+}$ cells in bone marrow and peripheral blood; however, ELP6 is not co-expressed with the high affinity IgE receptor (a basophil marker), showing that ELP6 is expressed on $IL5R^{+}$ eosinophils but not basophils. It is also possible that ELP6 is only expressed on a subset of eosinophils. Our results show that ELP6 is a myeloid marker in developing myeloid BMMC

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

and mature eosinophils and neutrophils. ELP6 is expressed in developing myeloid cells downstream of the CMP, as erythroid cells and lymphoid cells don't express ELP6.

ELP6 was initially identified as being transcriptionally upregulated by gene profiling in an IL-5 mediated model of eosinophil development; therefore, it is not surprising that ELP6 is expressed in IL5R positive subsets during granulopoiesis. The lack of detectable up-regulation of other elongator subunits in the initial gene profiling experiment may be due to the small three-fold increase in transcriptional expression of ELP6 or perhaps other subunits are regulated posttranscriptionally. Furthermore, although ELP6 transcripts are ubiquitously expressed in human tissue mRNA, not all tissue lysates express ELP6 protein, suggesting either post-transcriptional regulation or degradation of the la-bile ELP6 protein.

ELP6, a subunit of the Elongator holoenzyme, has recently been identified as the protein corresponding to c3orf75 [1]. Loss of elongator subunit, elp3, has been shown to decrease hemocyte development in drosophila, suggesting an analogous role for other Elongator subunits in human myeloid cells. ELP6 has been reported to be in the cytoplasm, where wobble tRNA modification [21] and tubulin acetylation occur. ELP6 has been identified as having a function in neuronal and cancer cell migration due to tubulin acetylation; therefore, expression of ELP6 in granulocyte cytoplasm may have a specific role in granulocyte cell migration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH grants 5R21AI07 9364-02 and 5R01AI009728-37. Special thanks to the Flow cytometry core facility, including Chris Leukel and the Fluorescence Microscopy Facility, including Chris Rodesch.

References

[1]. Close P, Gillard M, Ladang A, Jiang Z, Papuga J, Hawkes N, Nguyen L, Chapelle JP, Bouillenne F, Svejstrup J, Fillet M, Chariot A. DERP6 (ELP5) and C3ORF75 (ELP6) Regulate Tumorigenicity and Migration of Melanoma Cells as Subunits of Elongator. The Journal of Biological Chemistry. 2012; 287:32535–32545. [PubMed: 22854966]

[2]. Glatt S, Seraphin B, Muller CW. Elongator: Transcriptional or translational regulator? Transcription. 2012; 3

[3]. Winkler GS, Kristjuhan A, Erdjument-Bromage H, Tempst P, Svejstrup JQ. Elongator is a histone H3 and H4 acetyltransferase important for normal histone acetylation levels *in vivo*. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99:3517–3522. [PubMed: 11904415]

[4]. Solinger JA, Paolinelli R, Kloss H, Scorza FB, Marchesi S, Sauder U, Mitsushima D, Capuani F, Sturzenbaum SR, Cassata G. The Caenorhabditis elegans Elongator complex regulates neuronal alpha-tubulin acetylation. PLoS Genetics. 2010; 6:e1000820. [PubMed: 20107598]

[5]. Chen C, Tuck S, Bystrom AS. Defects in tRNA modification associated with neurological and developmental dysfunctions in Caenorhabditis elegans elongator mutants. PLoS Genetics. 2009; 5:e1000561. [PubMed: 19593383]

[6]. Versees W, De Groeve S, Van Lijsebettens M. Elongator, a conserved multitasking complex? Molecular Microbiology. 2010; 76:1065–1069. [PubMed: 20398217]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

[7]. Svejstrup JQ. Elongator complex: How many roles does it play? Current Opinion in Cell Biology. 2007; 19:331–336. [PubMed: 17466506]

[8]. Zhou X, Hua D, Chen Z, Zhou Z, Gong Z. Elongator mediates ABA responses, oxidative stress resistance and anthocyanin biosynthesis in Arabidopsis. The Plant Journal: For Cell and Molecular Biology. 2009; 60:79–90. [PubMed: 19500300]

[9]. Glatt S, Letoquart J, Faux C, Taylor NM, Seraphin B, Muller CW. The Elongator subcomplex Elp456 is a hexameric RecA-like ATPase. Nature Structural and Molecular Biology. 2012; 19:314–320.

[10]. Krogan NJ, Greenblatt JF. Characterization of a six-subunit holo-elongator complex required for the regulated expression of a group of genes in Saccharomyces cerevisiae. Molecular and Cellular Biology. 2001; 21:8203–8212. [PubMed: 11689709]

[11]. Lin Z, Zhao W, Diao W, Xie X, Wang Z, Zhang J, Shen Y, Long J. Crystal structure of elongator subcomplex Elp4-6. The Journal of Biological Chemistry. 2012; 287:21501–21508. [PubMed: 22556426]

[12]. Gilbert C, Kristjuhan A, Winkler GS, Svejstrup JQ. Elongator interactions with nascent mRNA revealed by RNA immunoprecipitation. Molecular Cell. 2004; 14:457–464. [PubMed: 15149595]

[13]. Chen YT, Hims MM, Shetty RS, Mull J, Liu L, Leyne M, Slaugenhaupt SA. Loss of mouse Ikbkap, a subunit of elongator, leads to transcriptional deficits and embryonic lethality that can be rescued by human IKBKAP. Molecular and Cellular Biology. 2009; 29:736–744. [PubMed: 19015235]

[14]. Miskiewicz K, Jose LE, Bento-Abreu A, Fislage M, Taes I, Kasprowicz J, Swerts J, Sigrist S, Versees W, Robberecht W, Verstreken P. ELP3 controls active zone morphology by acetylating the ELKS family member Bruchpilot. Neuron. 2011; 72:776–788. [PubMed: 22153374]

[15]. Wynshaw-Boris A. Elongator bridges tubulin acetylation and neuronal migration. Cell. 2009; 136:393–394. [PubMed: 19203572]

[16]. Huang B, Johansson MJ, Bystrom AS. An early step in wobble uridine tRNA modification requires the Elongator complex. RNA. 2005; 11:424–436. [PubMed: 15769872]

[17]. Walker J, Kwon SY, Badenhorst P, East P, McNeill H, Svejstrup JQ. Role of elongator subunit Elp3 in Drosophila melanogaster larval development and immunity. Genetics. 2011; 187:1067–1075. [PubMed: 21288872]

[18]. Holloway BA, Gomez de la Torre Canny S, Ye Y, Slusarski DC, Freisinger CM, Dosch R, Chou MM, Wagner DS, Mullins MC. A novel role for MAPKAPK2 in morphogenesis during zebrafish development. PLoS Genetics. 2009; 5:e1000413. [PubMed: 19282986]

[19]. Wayner EA, Hoffstrom BG. Development of monoclonal antibodies to integrin receptors. Methods in Enzymology. 2007; 426:117–153. [PubMed: 17697883]

[20]. Wagner LA, Christensen CJ, Dunn DM, Spangrude GJ, Georgelas A, Kelley L, Esplin MS, Weiss RB, Gleich GJ. EGO, a novel, noncoding RNA gene, regulates eosinophil granule protein transcript expression. Blood. 2007; 109:5191–5198. [PubMed: 17351112]

[21]. Hopper AK, Phizicky EM. tRNA transfers to the limelight. Genes and Development. 2003; 17:162–180. [PubMed: 12533506]

[22]. Lee JJ, Dimina D, Macias MP, Ochkur SI, McGarry MP, O'Neill KR, Protheroe C, Pero R, Nguyen T, Cormier SA, Lenkiewicz E, Colbert D, Rinaldi L, Ackerman SJ, Irvin CG, Lee NA. Defining a Link with Asthma in Mice Congenitally Deficient in Eosinophils. Science. 2004; 305:1773–1776. [PubMed: 15375267]

[23]. Manz MG, Miyamoto T, Akashi K, Weissman IL. Prospective isolation of human clonogenic common myeloid progenitors. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99:11872–11877. [PubMed: 12193648]

[24]. Mori Y, Iwasaki H, Kohno K, Yoshimoto G, Kikushige Y, Okeda A, Uike N, Niiro H, Takenaka K, Nagafuji K, Miyamoto T, Harada M, Takatsu K, Akashi K. Identification of the human eosinophil lineage-committed progenitor: revision of phenotypic definition of the human common myeloid progenitor. The Journal of Experimental Medicine. 2009; 206:183–193. [PubMed: 19114669]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

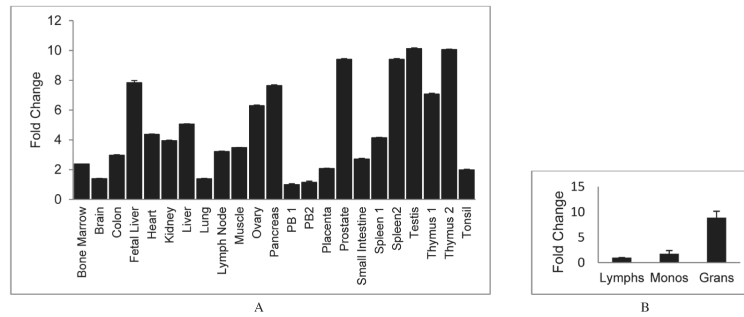

Fig. 1.

Real time quantitative RT-PCR of first strand cDNA using primers specific to ELP6. RNA is derived from: A. pooled normalized human tissue (Clontech, Mountain View, CA) B. human BMMC sorted by CD45 and side scatter into lymphocytes ($CD45^{high}$, $scatter^{low}$), monocytes ($CD45^{medium}$, $sidescatter^{medium}$) and granulocytes ($CD45^{low}$, side $scatter^{high}$). Standard error of triplicate PCR is shown.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

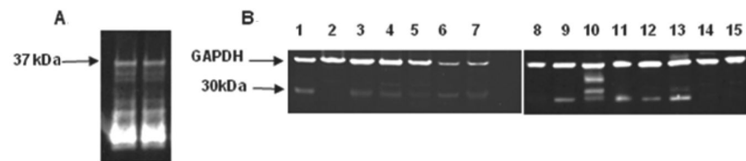

Fig. 2.

Western blots of human tissue lysates probed with anti-ELP6 (P5A7). A. Recombinant purified human ELP6. B. Lanes 1; bone marrow, 2; brain, 3; heart, 4; kidney 5; liver, 6; peripheral blood leukocyte, 7; thymus 8; brain 9; colon 10; esophagus (may be degraded) 11; lung 12; pancreas 13; spleen 14; uterus 15; testis. Arrows show molecular weight in kDa.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

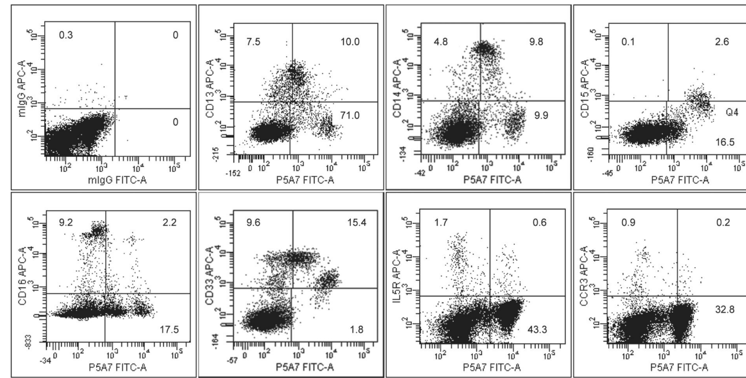

Fig. 3.

Flow cytometry of human BMMC. Cells were intracellularly stained with anti-*ELP6*-FITC (P5A7-FITC) or negative isotype controls (x axis) and other APC labeled antibodies (y axis) and analyzed on a Becton Dickinson FACSCanto2 as shown above. Percentages are shown.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

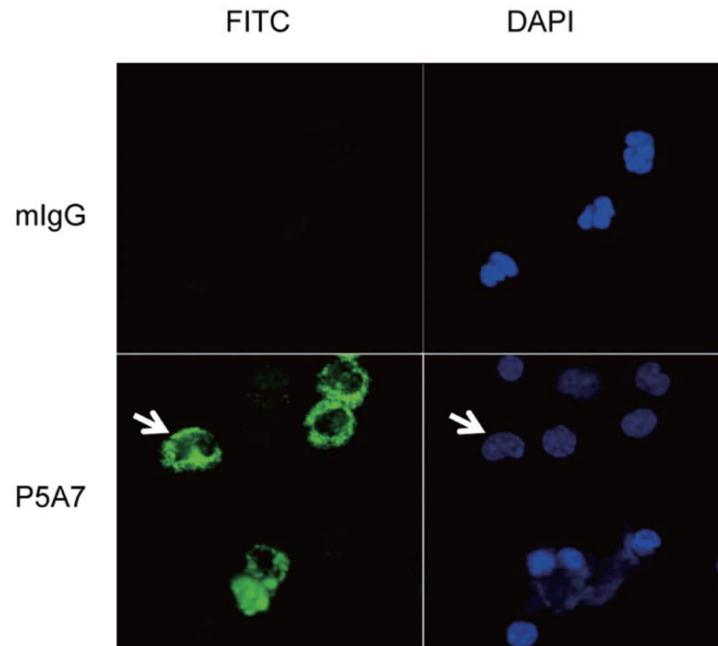

Fig. 4.

Confocal microscopy of cytospun BMMC labeled with mIgG-FITC (green, column 1, row 1) or anti-*ELP6* (green, column 1 row 2) and DAPI (blue, column 2) and photographed on a FV-1000-XY inverted confocal microscope. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/HAB-130268)

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

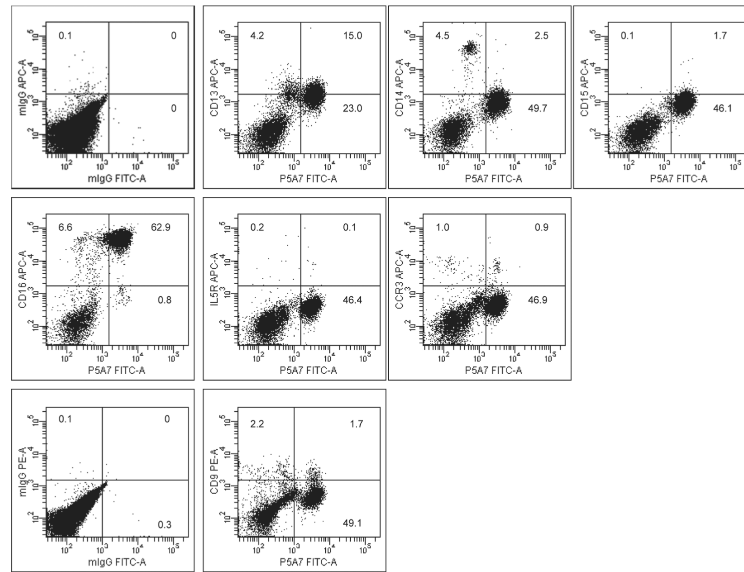

Fig. 5.

Flow cytometry of human buffy coats. Cells were intracellularly stained with anti-*ELP6*-FITC (P5A7-FITC) or negative isotype controls (x axis) and other APC labeled antibodies (y axis) and analyzed on a Becton Dickinson FACSCanto2 as shown above. Percentages are shown.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 1

BMMC were stained with anti-ELP6-FITC and other markers

	Experiment 1		Experiment 2		
Marker	**ELP6^{dim}**	**ELP6^{bright}**	**ELP6^{dim}**	**ELP6^{bright}**	**Cell type**
CD3	−	−	−	−	T cell
CD9	+	−	+	−	Eos, baso
CD11B	+	+	++	+	Gran, mono, NK, B_{sub}, T_{sub}, DC
CD13	++	+	++	−	Gran, mono
CD14	++	−	++	+	Mono, mac, gran (dim)
CD15	−	++	+	++	Gran
CD16		+	++	+	Neutrophils
CD19	−	−	−	−	B cells
CD33	++	+	+		Promyelocyte, myelocyte
CD34	+	−	+	−	Progenitor cell
CD38	+	−	+	−	Progenitor cell
CD45	++	+	++	+	Pan leukocyte
CD56	−	−	−	−	NK, T subset
IL5R	−	+	+	+	Eos, baso
CCR3	−	+	−	+	Eos. baso
FcεRI	−	−	n.d.	n.d.	Baso
CD235	−	−	−	−	Erythroid
c-kit	−	−	n.d.	n.d.	Stem cell
HLADR	+	-	n.d.*	n.d.*	Stem cell

++/+ indicates cell numbers.

* n.d. not done, n.p. not present. CCR3 is the eotaxin receptor. The results of two flow cytometry experiments are shown.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 2

Identification of sorted (90% pure) $ELP6^{-}$, $ELP6^{dim}$, and $ELP6^{bright}$ BMMC by light microscopy. Average of two experiments are shown in percentages

Cell type %	$ELP6^{-}$	$ELP6^{dim}$	$ELP6^{bright}$
Pronormoblast	0.2	0.0	0.0
Basophilic normoblast	1.5	0.4	0.5
Polychromatophilic normoblast	54.6	3.7	8.8
Orthochromic normoblast	10.0	0.7	2.1
Myeloblast	0.0	0.3	0.3
Promyelocytes	0.0	2.0	2.4
Myelocytes	0.3	36.0	27.6
Metamyelocytes	0.7	29.8	32.5
Bands	0.5	9.2	11.4
PMN	0.0	2.0	3.0
Monocytes	1.9	11.1	4.8
Plasma cells	0.1	0.0	0.4
Eosinophils	0.0	0.1	0.0
Basophils	0.0	0.1	0.0
Lymphocytes	30.2	4.5	6.3

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 3

The results of two flow cytometry experiments. Cells from buffy coats were stained with anti- ELP6-FITC and other markers

Marker	Experiment 1		Experiment 2		Cell type
	ELP6^{dim}	ELP6^{bright}	ELP6^{dim}	ELP6^{bright}	
CD3	–	–	–	–	T cell
CD9	+	+	+	+	Eos, baso
CD11B	+	++	n.p.	+	Gran, mono, NK, B_{sub}, T_{sub}, DC
CD13	+	+	+	++	Gran, mono
CD14	++	–	++	–	Mono, mac, gran(dim)
CD15	–	++	–	+	Gran
CD16	–	++	+	++	Neutrophils
CD19	–	–	–	–	B cell
CD45	+	++	–	++	Pan leukocyte
CD56	–	–	–	–	NK, T_{sub}
IL5R (IL5R)	+	+	+	+	Eos, baso
CCR3	–	+	–	+	Eos, baso
FcεRI	-	-	n.d.**	n.d.**	Baso

++/+ indicates cell numbers

* n.d. not determined.

** n.p. not present.

– is negative.