Progress in Hematology

Controlling α-globin: a review of α-globin expression and its impact on β-thalassemia

Hsiao Phin Joanna Voon and Jim Vadolas

Cell and Gene Therapy Research Group, The Murdoch Children's Research Institute, The University of Melbourne, Royal Children's Hospital, Parkville, Melbourne, Australia

ABSTRACT

Synthesis of α-globin and α-globin subunits of hemoglobin occurs at high levels during erythrocyte differentiation in a tightly controlled and co-ordinated fashion. Expression of α-globin is a fascinatingly complex process which has been meticulously defined in several recent studies, from chromatin modifications to Pol II recruitment. Following this, α-globin transcripts are processed and stabilized by a protein complex which binds the 3' untranslated region. Transcription and stabilization contribute to high level expression of α-globin. However, translation of α-globin at levels exceeding α-globin expression damages cellular membranes and results in β-thalassemia. It is, therefore, crucial that α-globin proteins are properly folded and stabilized, processes which are dependent on the presence of haem and AHSP. The exceedingly well-characterized process of α-globin expression elegantly illustrates the complex interaction of factors which are required to balance necessary high expression against the negative impacts of overexpression.

Key words: hemoglobin, α-globin, thalassemia.

Citation: Voon HPJ, and Vadolas J. Controlling α-globin: a review of α-globin expression and its impact on β-thalassemia. Haematologica 2008; 93:1868-1876. doi: 10.3324/haematol.13490

©2008 Ferrata Storti Foundation. This is an open-access paper.

©Ferrata Storti Foundation

Introduction

Hemoglobin is the major oxygen carrying component in blood and is vital to the survival of most multicellular animals. In higher order vertebrates, this essential protein is a tetramer composed of two related though distinct globin chains. The β-globin gene family is located on human chromosome 11 in a region which is tightly condensed and transcriptionally silent in all non-erythroid cells. In contrast, the α-like genes are located on human chromosome 16 in a region which encodes many housekeeping genes.[1] Though segregated onto separate chromosomes and located in distinctly different chromosome environments, both gene clusters nonetheless display the same, strictly regulated pattern of expression during whole organism development and individual erythrocyte differentiation. Both clusters are arrayed 5' to 3' in order of developmental expression and both are synthesized in a co-ordinated fashion at extremely high levels during erythroid differentiation.[2]

It is crucial to erythrocyte formation that the two globin chains are produced at balanced levels since any disruptions resulting in an excess of either chain has significant, deleterious effects on red cell survival, a situation which is most clearly illustrated by the thalassemias.[2] The thalassemias are commonly inherited hemoglobinopathies characterized by reduced hemoglobin synthesis and affects an estimated 7% of the world population. β-thalassemia arises due to mutations which result in reduced β-globin expression and excess, unbound α-globin chains. When both copies of β-globin are defective, excess α-globin chains form large insoluble precipitates in the cell causing membrane damage and resulting ultimately in premature cell death.[3]

Reduced expression of α-globin in the context of β-thalassemia can lead to considerable phenotypic improvements and α-globin is the most well-defined modifier gene known to impact on severity of β-thalassemia.[2] In addition, the regulation and expression of α-globin has been closely examined at all levels, from genes to protein. The combination of these factors makes this protein an attractive target for potential therapeutic interventions and gaining a comprehensive overview of α-globin and its interacting partners is essential to this process.

Acknowledgments: the authors are especially indebted to Radiomarathon Australia, The Greek Conference and the Greek and Cypriot communities of Melbourne, Australia for their continued efforts to advance our research and find a cure for β-thalassemia.
Funding: this work was supported by the National Health and Medical Research Council, the Murdoch Children's Research Institute and the Thalassaemia Society of Victoria.
Manuscript received June 12, 2008. Revised version arrived July 22, 2008. Manuscript accepted August 8, 2008.
Correspondence: Hsiao Phin Joanna Voon, Cell and Gene Therapy Research Group, The Murdoch Children's Research Institute, The University of Melbourne, Royal Children's Hospital, Flemington Road, Parkville 3052, Melbourne, Australia. E-mail: joanna.voon@mcri.edu.au

α-globin gene locus

The human α-globin cluster is located near the telomeric end of the short arm of chromosome 16. This cluster spans 26 kb and includes 3 functional α-like protein coding genes (ζ, α2 and α1), 2 expressed genes with unknown function (μ and θ1) and 3 pseudogenes (ψζ1, ψα1 and ψρ) (Figure 1A).[1] The three pseudogenes all carry inactivating mutations and are, therefore, not expressed. Until recently, the μ-globin gene was erroneously classed as a pseudogene (ψα2). However, Goh *et al*. were able to demonstrate transcription of this gene from a microarray analysis of adult reticulocytes and cord blood samples.[4] Expression of μ-globin was higher than θ or ζ but much lower than α-globin. Like θ-globin, μ-globin demonstrates a highly regulated pattern of expression with no detectable transcription in non-erythroid tissues. Both transcripts also yield no detectable protein product and deletions of θ or μ have no reported effects on clinical phenotypes so their function remains undefined.[4] Of the highly expressed functional α-genes, ζ-globin constitutes the embryonic α-gene while α2 and α1 are the dominant α-genes throughout all other stages of development.[5] Expression of ζ-globin is limited to the yolk-sac of primitive erythroblasts then silenced in the foetal liver concordant with the selective induction of α-globin expression.[5] This globin switch is dependent on two major factors; the transcriptional inactivation of ζ-globin[6] and the selective destabilization of ζ-globin mRNA in fetal and adult erythroblasts.[7,5] Though α2 and α1 encode an identical protein, α2 is expressed at levels 2-3 times higher than α1 at both the transcriptional and translational level.[8,9] The embryonic and adult α-genes share 58% homology at the amino acid level[1] and it appears that ζ-globin can be exchanged for α-globin in an adult organism without significant deleterious effects.[10] Interestingly, exchanging ζ-globin for α-globin in mice expressing an abnormal sickle β-globin gene, prevents erythrocyte sickling and improves the phenotype.[10]

α-globin regulatory elements

The α-globin regulatory elements include four conserved DnaseI HS sites which lie between 10 and 50 kb upstream from the start of the ζ-globin gene (Figure 1A).[11,12] A recent study by De Gobbi *et al*. utilized tiled microarrays to analyze a 220 kb region surrounding the α-like genes.[1] The study confirmed that the four known DnaseI HS sites bind erythroid-specific transcription factors and found no additional erythroid-specific regulatory elements in this region. Of these four sites (HS-10, HS-33, HS-40 and HS-48),[13,14] HS-40 forms the major regulatory element[15-17] and is the only element capable of directing high level expression of the α-globin gene.[18] Deletion of HS-40 from the human α-globin cluster leads to severe reductions in α-globin expression to <5% of normal.[17,19]

Analysis of the other elements in transgenic mice indicate that combinations of HS-10, HS-33 and HS-48 in small constructs are able to direct tissue and developmental stage specific expression but are unable to drive substantial levels of α-globin expression.[20] This has been further confirmed in a newly identified mutation in the enhancer which resulted in an α-thalassemia phenotype.[19] The mutation involved the deletion of a 16 kb region including the HS-40 and HS-48 while leaving HS-33 and HS-10 intact. Analysis of the affected chromosome indicated that α-globin expression was reduced to <1% of normal thus demonstrating that HS-33 alone or in combination with HS-10 has little or no positive effect on α-globin expression.[19] Furthermore, transgenic mice created with a human BAC containing only HS-48 but lacking all other conserved HS sites did not express any detectable levels of human α-globin at any stage during development, strongly indicating that HS-48 alone is also unable to drive substantial expression of α-globin.[21]

It is interesting that though these four HS sites are conserved between mouse (HS-31, HS-26, HS-21 and HS-8) and human, the deletion of the mouse HS-26 (homologous to human HS-40) only results in a 50% reduction in mouse α-globin expression.[22] Analysis of the mouse α-globin locus revealed the presence of an additional HS-12 element capable of binding the pentameric erythroid complex essential for activating α-globin transcription while the human homologous region lacks these critical binding sites.[13] In addition, the mouse α-globin promoter also binds GATA1, a crucial erythroid regulator, while the human promoter which lacks the consensus binding site, does not (Figure 1B).[13]

Transcriptional activation of α-globin

The binding of transcription factors in the pentameric erythroid complex to HS sites in the α-globin locus is

©Ferrata Storti Foundation

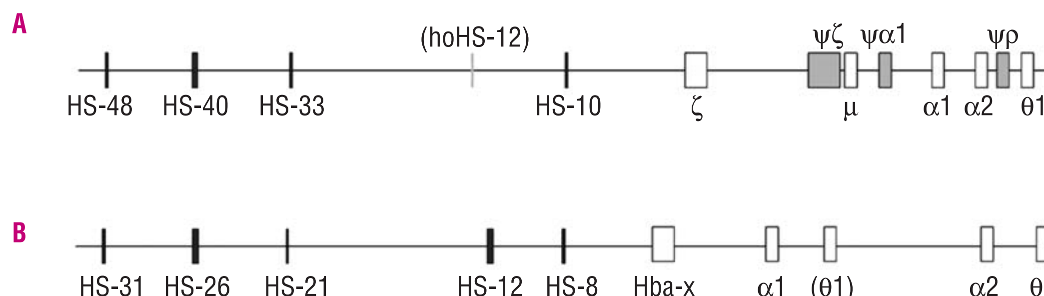

Figure 1. Schematic representation of the α-globin cluster. (A) Human α-globin cluster. The four conserved DnaseI hypersensitive sites known to bind erythroid transcription factors are located between 10-50 kb upstream of the start of the ζ-globin gene. The genes (and pseudogenes) of the α-globin cluster are arranged 5' to 3' in the order of developmental expression. (B) Murine α-globin cluster. Locus contains an additional DnaseI hypersensitive site (HS-12) which contributes to regulation of murine α-globin expression.

essential for high level expression of α-globin.[13,23] This complex (GATA-1, SCL, E2A, LMO-2 and Ldb-1) and other factors such as NF-E2, is thought to recruit Pol II to the α-globin locus then facilitate the transfer of Pol II to the promoters.[23] This is the final step in the activation of α-globin transcription which begins with a range of chromatin modifications. Priming of the α-globin cluster begins in multipotent hematopoietic progenitor cells.[24] In mouse cells, the four main erythroid specific HS sites appear at this stage of differentiation concurrent with initiation of histone H3 and H4 acetylation. As cells differentiate further down the erythroid lineage, a number of other sites also become hypersensitive to DNaseI, including the α-globin promoter, while the domain of acetylation extends to become fully developed in mature erythroblasts.[24]

In primary human erythroblasts, GATA-1, SCL and the entire pentameric erythroid complex were found bound to all four HS sites.[13] In addition to this, both subunits of the NF-E2 transcription factor were also enriched at HS-40 and to a lesser extent at HS-48.[13] Maximal binding of all transcription factors were detected in basophilic and polychromatophilic erythroblasts, coinciding with maximal levels of histone H3 and H4 acetylation.[13] At the onset of transcription, the promoter then becomes bound by SP/X-Kruppel-like transcription factors (Sp/X-KLF).[23] These factors behave as activators in erythroid cells and are known to interact with GATA-1. At this stage, the pre-initiation complex (PIC), which includes general transcription factors and Pol II, is also detectable at the remote HS sites and is highly enriched at the promoter.[23]

In the absence of upstream HS elements, PIC binding to α-globin promoter is greatly reduced suggesting that recruitment of the full PIC to the promoter is heavily dependent on upstream elements.[23] Studies on the β-globin locus indicate that Pol II is transferred to the promoter in an NF-E2 dependent manner, though the mechanism of transfer is unknown.[13] However, in cells expressing α-globin, there is a detectable increase in interactions between upstream regulatory elements and the α-globin genes.[23] These results seem to suggest that there is a change in chromosome conformation which results in physical interaction between the HS elements and the α-globin promoter, and this interaction facilitates the delivery of activated Pol II from the enhancer elements to the promoter.

α-globin mRNA

Once the enhancer regions have co-ordinated the positioning of Pol II on the promoter, transcription of α-globin begins and transcripts rapidly accumulate. Active transcription occurs mainly in the basophilic erythroblast, the first committed erythroid precursor, and in the polychromatophilic erythroblast.[25] By the next stage of differentiation, in orthochromatophilic erythroblast, the cellular chromatin has completely condensed and transcription has ceased as the cell prepares to extrude its nucleus prior to full maturation. It is interesting that although transcription of α-globin occurs only during the early stages of erythroid cell maturation, α-globin protein is synthesized for an additional 4-6 days after

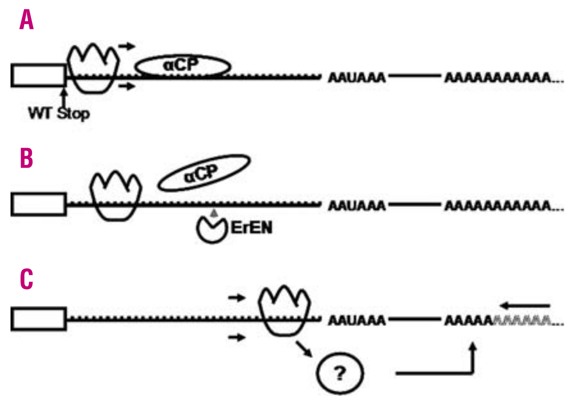

Figure 2. Disruption of α-globin mRNA stability. (A) Ribosomes extend past the WT stop codon in anti-termination mutants (e.g. α^{cs}) and trigger destruction of α-globin transcripts. (B) Ribosomal displacement of αCP RNP complexes expose an ErEN cleavage site and result in premature cleavage of transcripts. (C) Ribosomes extending into codon 14-19 past the WT stop codon trigger a second destabilization event resulting in accelerated deadenylation via an unknown mechanism.

transcriptional silencing of erythropoietic progenitor cells.[25] This phenomenon is dependent on the high level stability of α-globin mRNA transcripts, with half-life of α-globin mRNA estimated to be greater than 24 hours in erythroid cells.[5,25,26]

The importance of α-globin mRNA stability can be illustrated in cases of α-thalassemia resulting from α-Constant Spring (α^{CS}) mutations. The α^{CS} mutation occurs in the dominant α2 gene and involves the substitution of the translational stop codon to a glutamine codon (UAA→CAA).[25,27] This results in translation of an additional 31 codons of the 3' untranslated region (UTR) and produces low levels (3% of WT) of a structurally abnormal α-globin chain.[25,27] Experiments in transgenic mice indicated that the α^{CS} mRNA was four-fold less stable than α^{WT} mRNA and this appeared to be linked to a shortened poly(A) tail in α^{CS} transcripts.[28] It appears that there are two independent mechanisms, both erythroid specific, which reduce stability of α^{CS} mRNA. The first involves the ribosomal displacement of an RNA-protein (RNP) complex, bound to the α-globin 3'UTR, which stabilizes α-globin transcripts.[29-33] In the second, it appears that ribosome extension far into the 3'UTR triggers accelerated deadenylation of the α^{CS} transcript (Figure 2).[34]

This new mechanism, termed *ribosome-extension mediated decay* (REMD) is thought to be related to more general mRNA surveillance machinery such as nonsense mediated decay or the non-stop decay pathway.[34] Transcripts harboring the α^{CS} mutation are translated past the WT stop codon and into the 3' UTR before terminating at the poly(A) signal.[35] When a series of constructs which allowed the ribosome to extend incrementally into the α-globin 3'UTR were tested, Kong *et al*. discovered a second destabilization event occurred when the ribosome extended between 14-19 codons past the WT stop codon.[34] They postulated that this could be due to interference with cytoplasmic functions

©Ferrata Storti Foundation

of the AAUAAA determinant or disruption of crucial RNP complexes at 3' terminus of mRNA important in maintenance of poly(A) tails, therefore resulting in accelerated deadenylation. Interestingly, this REMD pathway did not appear to be active in transfected non-erythroid HeLa cells stably expressing α-globin, perhaps reflecting a bias towards only highly stable transcripts in erythroid cells. Alternatively, it may also be possible that ribosomal extension disrupts a second erythroid stabilizing complex, not unlike the α-complex.

The α-complex binds to three C-rich regions located at position 29-70 in the 3'UTR and deletions or mutations in this region which prevent α-complex formation significantly reduce the half-life of α-globin transcripts. The selective binding of the α-complex is mediated by two distinct but similar poly(C) binding proteins, αCP1 and αCP2.[31] The αCP proteins are ubiquitously expressed in human and mouse cells but appear to have different roles in other cell types.[25] In erythroid cells, αCP nucleates the formation of an α-complex which protects the α-globin transcript from premature degradation by an erythroid enriched endoribonuclease[36] and helps stabilize the poly (A) tail thus prolonging the half-life of the transcript.[24] In addition to stabilizing mRNA, αCP also appears to have a nuclear role in the splicing of pre-mRNA α-globin transcripts. It was recently discovered that αCPs are loaded onto α-globin transcripts in the nucleus and in addition to the 3'UTR, also bind a C-rich region in intron 1.[37] In HeLa cells selectively depleted of αCP, splicing efficiency of a truncated α-globin construct (comprising exon 1, intron 1 and exon 2) was increased approximately four-fold from 15% to 58%. Thus, it appears that nuclear αCP binding to intron 1 represses splicing of the α-globin transcript. Conversely, nuclear binding of αCP to the 3'UTR appears to enhance splicing of the α-globin pre-mRNA.[37] Experiments were conducted in HeLa and MEL cells comparing WT α-globin and a mutated transcript with a modified 3'UTR which does not bind αCP (αΔPR). In both cell types, it was found that WT transcripts were spliced with higher efficiency than mutated αΔPR transcripts.[37] Given the apparently contradictory roles of αCP binding in different regions of α-globin transcripts, it may be interesting to determine if the affinity of αCP for each site varies with stages of cell differentiation. It is tempting to speculate that late in differentiation, αCP bound to the 3'UTR enhances splicing and stabilizes α-globin transcripts in preparation for high levels of hemoglobin synthesis. However, in early stages of α-globin expression, αCP may bind to intron 1 and repress splicing and expression until the appropriate time. This could then potentially represent another level of control at the transcriptional stage, preventing inappropriate expression of α-globin which would be deleterious to the developing erythrocyte.

The α-globin protein

The transcriptional process gives rise to an estimated 10,000-20,000 mature, stabilized transcripts which are utilized repeatedly to generate an estimated 280 million molecules of complete hemoglobin molecules per cell.[24] In adults, each hemoglobin molecule comprises four subunits, two β-globin chains and two chains of α-globin, each coordinating one molecule of haem. The ferrous iron of the haem is linked to proximal (F8) histidine while the porphyrin ring is wedged in place by a phenylalanine on the α-globin (or β-globin) chain. In functional hemoglobin, oxygen slips freely between a second, distal (E7) histidine in the haem pocket and the ferrous iron during oxygenation and deoxygenation. Occasionally, the ferrous iron binds spontaneously to the distal histidine and is oxidized to ferric iron forming methemoglobin (metHb) which is without value as a respiratory pigment. This reaction occurs in an estimated 1% of total circulating haemoglobin every day but a series of protective mechanisms exist to reconvert the pigment back to functional hemoglobin.

Role of haem in protein folding

The correct co-ordination of the haem molecule is, of course, essential to the function of hemoglobin but in addition to this, haem binding also appears to stabilize the globin chains and promote proper folding of the polypeptide.[38] *In vitro* studies using a wheat germ cell-free translation system demonstrated that nascent α-globin chains with as few as 86 amino acid residues are able to associate co-translationally with haem during its synthesis on the ribosome.[38] This suggests that haem binding to nascent α-globin chain may stabilize the growing polypeptide on the ribosome and promote the formation of the native tertiary structure of α-globin.

Interestingly, α-globin has been demonstrated to have a ten-fold greater ability to bind haem compared to β-globin[38] and may actually facilitate haem integration into apo-β-globin (and γ-globin) chains. In a separate set of *in vitro* experiments, Adachi *et al*. utilized an assay system capable of distinguishing between haem-incorporated and apo-globin monomers, dimers or heterotetramers to assess the effects of haem addition on hemoglobin formation. The authors demonstrated that in the absence of any additional haemin, the addition of haem-containing α-globin (α^h) to newly synthesized apo-β-globin chains resulted in formation of approximately 50% haem-containing $\alpha^h\beta^h$ heterodimers.[39] In contrast, in the reverse reaction, where haem-containing β-globin (β^h) were added to newly synthesized apo-α-globin chains, the majority of heterodimers formed were semi-haem containing $\alpha^0\beta^h$ and only trace amounts of $\alpha^h\beta^h$ heterodimers were observed.[39]

It is also interesting to note that apo-γ-globin appears to be more dependent on the presence of haem-containing α-globin compared to apo-β-globin. Addition of α^h chains to newly synthesized apo-γ-globin led to the formation of haem-containing heterodimers $\alpha^h\gamma^h$ but co-translation of α-globin and γ-globin in the presence of haemin did not result in any detectable αγ dimers. This suggests that the presence of properly folded haem-containing α-globin chains are critical to the formation of αγ dimers.[39] Overall, these results suggest that haem-containing α-globin associates with nascent apo-β-globin (or γ-globin) chains leading to stabilization and promoting haem incorporation in the β-globin or γ-globin chain.[39,40] This hypothesis is further supported by the detection of $\alpha^h\beta^0$ intermediates prior to the formation of

©Ferrata Storti Foundation

$\alpha^h\beta^h$ dimers *in vivo*[41] and by the presence of unbound α-globin chains known to exist in erythroid cells.[42,43]

α-hemoglobin stabilizing protein

As free α-globin is an unstable, reactive molecule capable of destroying erythroid progenitor cells, this small pool of excess α-globin chains must be stabilized to limit its reactivity. This is achieved by the binding of an abundant erythroid-expressed protein known as α-hemoglobin stabilizing protein (AHSP). This protein was identified in a screen for proteins regulated by GATA-1 and was found to bind to α-globin specifically.[44] AHSP is able to interact with multiple forms of α-globin including apo-, ferrous and ferric states bound to a variety of ligands[45] and reduces oxidant-induced precipitation of α-globin in solution.[44,45] AHSP binds α-globin at the α1β1 dimer interface, opposite the haem binding pocket, with lower affinity than β-globin and is easily displaced by β-globin binding.[45]

The initial binding of AHSP to free oxy-α-globin results in a number of structural changes including displacement of the proximal F8 histidine and co-ordination of the ferrous iron by the distal E7 histidine.[45-47] The oxygen molecule also binds to the proximal side of the haem group resulting in an overall structure which exposes the oxygen binding site.[46,47] This initial interaction is fully reversible and β-globin can readily displace AHSP to generate functional HbA under reducing conditions.[46]

However, if oxy-α-globin remains bound to AHSP, the exposure of the oxygen molecule predisposes it to the spontaneous loss of HO_2 and rapid auto-oxidation of the haem iron occurs. This reaction was shown to be oxygen dependent[46] and results in a formation of a second, discrete, ferric bis-histidyl complex.[47] In this state, the haem iron is oxidized to Fe(III) and becomes co-ordinated by both the proximal and the distal histidines.[45,48] This hemichrome structure is more resistant to denaturation and haemin loss compared to oxidized free α-globin and inhibits the reaction of Fe(III) α-globin with oxidants such as hydrogen peroxide.[46]

Both the ferrous and the ferric form of AHSP bound α-globin are capable of binding to oxy-β-globin to form adult hemoglobin (HbA) tetramers, but the ferric form results in HbA products with reduced electrophoretic mobility.[46] In addition, HbA containing Fe(III) α-globin was rapidly converted to cyanomet Hb upon exposure to cyanide while HbA generated from AHSP modified α-globin was resistant.[46] Together, these findings suggest that the AHSP-mediated rearrangement of α-globin to the bis-histidyl structure is retained upon binding to β-globin and the α-subunits retained a hemichrome formation which inhibits cyanide binding and alters the electrophoretic mobility. However, this alteration appears to be reversible as the AHSP-induced hemichrome formation can be reduced back to ferrous iron under appropriate conditions, generating functional hemoglobin capable of binding oxygen.[46] Therefore, AHSP can sequester α-globin in a reversible inert state if β-globin is unavailable or, alternatively, it can bind α-globin transiently during normal HbA formation.

In fact, it appears that AHSP binding to α-globin actually facilitates the formation of HbA tetramers *in vitro*. Using a wheat germ-derived transcription and translation to express α-globin, it was found that addition of β-globin with AHSP augmented the formation of HbA in a dose-dependent fashion.[49] This is consistent with previous observations that soluble haem-containing α-globin chains associate with apo-β-globin to promote formation of HbA,[39] and AHSP maintains the newly synthesized α-globin in a soluble state to facilitate this interaction.[48] In addition, similar to haem, AHSP also acts as a molecular chaperone to promote folding of nascent apo-α-globin. AHSP was shown to stabilize α-globin *in vitro*, rendering it resistant to protease digest in a haem-independent manner.[49] Consistent with the role of AHSP as a molecular chaperone, AHSP is also able to promote refolding of denatured α-globin.[49] As AHSP and haem bind α-globin at different sites, the two molecules most likely synergize to stabilize the native structure to facilitate interaction with β-globin.

AHSP functions in vivo

These roles for AHSP are supported by the *in vivo* effects of AHSP loss. AHSP (–/–) homozygous knockout mice exhibit mild hemolytic anemia, shortened erythrocyte lifespan and high levels of reactive oxygen species (ROS) consistent with the presence of unstable α-globin.[44] These erythrocytes also display prominent eosinophilic inclusions (Heinz bodies) which contain both α-globin and β-globin precipitates.[44,48,50] It was originally hypothesized that reduction of α-globin synthesis may ameliorate the AHSP phenotype since there would be lower levels of excessive toxic α-globin. However, surprisingly, the loss of a single α-globin gene (-+/++) in a AHSP (–/–) null background resulted in significantly more anemic phenotype.[49]

The compound AHSP (–/–) α-KO (-+/++) mice were found to have significantly higher levels of both α-globin and α-globin lodged in erythrocyte membranes compared to heterozygous α-KO mice.[49] This finding supports the role of AHSP in stabilizing the free α-globin pool and therefore facilitating integration with α-globin prior to formation of HbA tetramers. The loss of AHSP reduces the pool of stable α-globin chains available for HbA formation. Under these conditions, further reduction of α-globin expression would result in even fewer α-globin chains, which would be unstable, and therefore increased free α-globin.

These results imply that mutant α-globin chains unable to bind AHSP may exhibit a more severe phenotype than simple loss of α-globin alone. At least two α-globin termination mutants, the previously described α^{CS} mutation[27] and α^{T} (stop codon→tyrosine substitution),[51] result in low levels of structurally abnormal α-globin products.[35,51] When tested in a yeast two-hybrid system, a β-galactosidase assay detected reduced α^{CS} and α^{T} interaction with AHSP compared to WT α-globin.[52] Further testing may be required to confirm this reduced interaction but if proven correct, it may help explain some of the features associated with α^{CS} or α^{T} related α-thalassemia. Both α^{CS}- and α^{T}- globins have been detected in the cell membrane and found associated with the membrane skeleton,[35,53] consistent with a

©Ferrata Storti Foundation

reduced ability to bind AHSP. It also appears that this abnormal interaction results in more severe anemia[35] than in deletional α2 thalassemias, similar to the AHSP (–/–) α-KO (-+/++) mice.

Predictably, the loss of AHSP in thalassemic heterozygous α-KO mice also results in a poorer phenotype and increased α-globin precipitation.[50] AHSP-KO, β-thalassemic mice had significantly impaired intrauterine survival and were approximately 20% more anemic than heterozygous α-KO mice with normal AHSP expression.[48,50] These studies in mice indicate that AHSP may be a possible modifier gene in human β-thalassemias. However, mutations which ablate AHSP in humans are rare[49,54] and investigations into possible effects of reduced AHSP expression in β-thalassemia have so far been largely inconclusive. Preliminary reports indicate that AHSP may be a modifier in the human population[48,55-57] but as yet, there has been little definitive evidence that this is the case.

Phenotypic effects of α-globin in β-thalassemia

In contrast to the situation with AHSP, a protein where function has only recently been defined, the interaction of α-globin mutations with β-thalassemia are exceedingly well characterized.[2,58-62] Though β-thalassaemia arises from reduced expression of β-globin leading to decreased formation of functional hemoglobin tetramers, this plays a relatively minor role in contributing to the severely anemic phenotype.[2,58,63] Instead, it is the damage caused at the cellular level by excess, improperly paired α-globin chains which leads to premature cell death and accounts for the majority of the pathology.[64]

Cellular consequences of excess α-globin chains

In the absence of normal β-globin production, it is likely that α-globin is synthesized at levels which exceed the binding capacity of available AHSP. Under these conditions, free α-globin is able to accumulate in red blood cells and precursors leading invariably to a variety of deleterious alterations. As previously noted, free α-globin is a highly unstable molecule and when devoid of stabilizing binding partners, forms large insoluble aggregates which can be visualized by light microscopy in an estimated one third of erythroid progenitor cells.[65]

Much of the excess α-globin undergoes auto-oxidation to produce equal molar ratios of metHb and superoxide[66] which can lead to an autocatalytic oxidative process in the cell. The oxidized ferric iron is bound more loosely to α-globin compared to the ferrous form which results in degradation of haem[67] and ultimately the release of iron which lodges in the cell membrane.[66,68] In addition, oxidized haem-containing α-globin is also found bound to the cytoskeleton.[64,69,70] In this unstable conformation, both the haem group and the iron are able to participate in redox reactions leading to generation of ROS which can then oxidize adjacent membrane proteins.[65] Several critical membrane proteins including Band 4.1, spectrin and transmembrane Band 3 have been found to be oxidised in β-thalassemic erythroid precursors, leading to severe membrane abnormalities and eventual hemolysis.[65,69]

The high levels of ROS production in these affected cells may also trigger apoptotic pathways leading to premature cell death in as many as 60-80% of erythroid progenitor cells.[71] In thalassemic erythroid precursor cells, this response appears to be mediated by the cell surface interactions of FAS with FAS-ligand, the death receptor pathway, since all Annexin V positive apoptotic cells were also FAS positive.[71] In addition, erythrocytes in peripheral circulation also bind increased levels of IgG.[66] Under normal conditions, IgG typically binds senescent cell antigens to signal macrophage removal,[72,73] but in β-thalassemia, this process appears accelerated by the accumulated oxidative stress and subsequent haem degradation and membrane damage.[66]

Thus, the excess α-globin in β-thalassemia generates high levels of ROS in an autocatalytic process which most likely leads to premature apoptosis in the bone marrow and ineffective erythropoiesis. In addition, red cells which survive to reach the circulation continue to be subjected to high levels of oxidative stress leading to structural abnormalities of the cytoskeleton and hemolysis or alternatively, accelerated senescence and macrophage clearance.

Co-inheritance of α-globin mutations in β-thalassemia

Given that excess production of α-globin leads to widespread detrimental effects in β-thalassemia, it logically follows that reduction of α-globin synthesis improves the β-thalassemic phenotype. Since the first report of α-globin as a potential modifier gene in β-thalassemia by Fessas *et al*. in 1961, innumerable studies have detailed the interactions of almost all theoretical possibilities for different combinations of α- and β-thalassemia genes.[2,58-60,74-78] However, most of these patient groups were relatively small and therefore wide-ranging conclusions about phenotypic effects for particular genotypes are not possible. Nevertheless, the overall picture which has emerged is quite clear; co-inheritance of α-thalassemia with β-thalassemia invariably improves clinical outcomes. The extent to which α-thalassemia alleles improves phenotype is dependent on the number of inactivated α-globin genes, the severity of β-globin mutations and most importantly, the degree to which globin imbalance is corrected. The clearest phenotypic improvements are generally observed where there is residual β-globin synthesis, as in cases of homozygous or compound heterozygous β^+ mutations. In these situations, inactivation of one α-globin gene has somewhat unpredictable outcomes but mutations in two α-globin genes tends to lead to a mild intermedia phenotype.[2] Inactivation of three α-globin genes has been known to completely balance globin synthesis ratios, even on a background of severe β-thalassemia alleles, and can confer transfusion independence.[79]

Conversely, the inheritance of extra copies of α-globin can also convert the normally clinically silent phenotype of heterozygous β-thalassemia to a clinically significant anemia.[55,80-85] In extreme cases where homozygous triplicated or quadruplicated α-globin are present, a relatively severe transfusion-dependent phenotype can

©Ferrata Storti Foundation

emerge.[80] Hence, alterations in α-globin synthesis have considerable effect on β-thalassemic phenotypes; increased α-globin expression in heterozygous β-thalassemia leads to greater imbalance and converts a silent carrier state to a clinically significant anemia while reduced α-globin synthesis in homozygous β-thalassemia reduces the severity of anemia by restoring globin balance.

Conclusions and future directions

The disrupted interactions between α-globin and β-globin in cases of thalassemia clearly illustrate the importance of correctly co-ordinated gene expression. In order to facilitate the survival and growth of a healthy organism, it is essential that large amounts of hemoglobin are synthesized in red blood cells yet this process must also be carefully controlled to prevent accumulation of excess chains. Defects in any one of these complex interactions invariably leads to impaired red cell survival and adverse phenotypic outcomes as illustrated by the thalassemias. In cases of β-thalassemia, the damage caused by unpaired α-globin chains is quite remarkable and clearly accounts for much of the pathology. As such, reducing α-globin gene expression may help ameliorate the phenotype of β-thalassemia.

A number of recent studies have attempted to use the RNA interference (RNAi) pathway to achieve this goal with some success.[86-88] It appears though, that delivery of effector molecules may prove problematic and given that α-globin mRNA is highly expressed, this may not represent the ideal target. However, there are certain key differences between α-globin and β-globin expression which may reveal other avenues of intervention. Most notably, α- and β-globin reside in distinct chromatin environments and are most likely marked with different epigenetic modifications. At least one protein is known to interact in *trans*, either directly or indirectly, with the α-globin but not the β-globin locus.

ATRX is a large nuclear localizing protein widely expressed throughout development which when mutated, results in an α-thalassemia-like phenotype in addition to a number of other defects.[89] ATRX belongs to the SWI/SNF2 protein family of chromatin remodelers and appears to play a role in DNA methylation.[90] This protein also seems to have a role at heterochromatin where it is possible that its presence may prevent silencing of α-globin during terminal differentiation of erythroid cells. However, the recent set of investigations at the α-globin gene locus using pioneering technologies failed to detect ATRX at this locus.

It seems that as thorough as these studies were, many other details remain to be discovered. In particular, it would be most interesting to discover the exact functions of epigenetic modifications at this locus and the role of ATRX or other factors in mediating these modifications. At the transcriptional level, the multifunctional roles of αCP have only recently been discovered and the regulation of αCP binding as well as the repertoire of interacting proteins remain undefined. Given the importance of α-globin stability in proper protein expression, developing a deeper understanding of regulating elements will doubtless prove invaluable in any attempts to manipulate gene expression.

At the protein level, the relatively recent discovery of AHSP has led to a number of highly insightful studies regarding the role of this crucial interacting partner though unsurprisingly, there remain many issues to be addressed. Of these, perhaps the subject of greatest interest is the role of AHSP in β-thalassemia as a potential modifier. However, mutations which ablate AHSP appear quite rare, a situation which might suggest that either heterozygous AHSP mutations, unlike the thalassemias, provide negligible selective advantage against malaria or that these mutations are highly deleterious.

Nonetheless, these combined studies have produced a clear outline of the control of α-globin expression, as well as an exceptional model for general mammalian gene expression. Hopefully, future studies will help to generate finer details leading to a more comprehensive understanding and potentially reveal novel avenues for therapeutic interventions.

Authorship and Disclosures

HV prepared the manuscript; JV critically revised the manuscript. All authors were involved in drafting the article and revising it critically for important intellectual content and have approved the final version to be published. The authors also declare no potential conflict of interest.

©Ferrata Storti Foundation

References

1. Higgs DR, Vickers MA, Wilkie AO, Pretorius IM, Jarman AP, Weatherall DJ. A review of the molecular genetics of the human α-globin gene cluster. Blood 1989;73:1081-104.
2. Weatherall DJ, Clegg J. The Thalassaemia Syndromes 4th Edition. London: Blackwell Science, 2001.
3. Schrier SL. Thalassemia: pathophysiology of red cell changes. Annu Rev Med 1994;45:211-8.
4. Goh SH, Lee YT, Bhanu NV, Cam MC, Desper R, Martin BM, et al. A newly discovered human α-globin gene. Blood 2005;106:1466-72.
5. Liebhaber SA, Russell JE. Expression and developmental control of the human α-globin gene cluster. Ann of N Y Acad Sci USA 1998;850:54-63.
6. Liebhaber SA, Wang Z, Cash FE, Monks B, Russell JE. Developmental silencing of the embryonic zeta-globin gene: concerted action of promoter and 3' flanking region combined with stage-specific silencing by the transcribed segment. Mol Cell Biol 1996; 16:2637-46.
7. Russell JE, Morales J, Makeyev AV, Liebhaber SA. Sequence divergence in the 3' untranslated regions of human ζ- and α-globin mRNAs mediates a difference in their stabilities and contributes to efficient α-to-ζ gene development switching. Mol Cell Biol 1998;18:2173-83.
8. Liebhaber SA, Cash FE, Ballas SK. Human α-globin gene expression. The dominant role of the α 2-locus in mRNA and protein synthesis. J Biol Chem 1986;261:15327-33.
9. Albitar M, Cash FE, Peschle C, Liebhaber SA. Developmental switch in the relative expression of the α1- and α2-globin genes in humans and in transgenic mice. Blood 1992;79: 2471-4.
10. He Z, Russell JE. Antisickling effects of an endogenous human α-like globin.

Nat Med 2004;10:365-7.
11. Hughes JR, Cheng JF, Ventress N, Prabhakar S, Clark K, Anguita E, et al. Annotation of cis-regulatory elements by identification, subclassification, and functional assessment of multispecies conserved sequences. Proc Natl Acad Sci USA 2005;102:9830-5.
12. Tufarelli C, Hardison R, Miller W, Hughes J, Clark K, Ventress N, et al. Comparative analysis of the α-like globin clusters in mouse, rat, and human chromosomes indicates a mechanism underlying breaks in conserved synteny. Genome Res 2004;14:623-30.
13. De Gobbi M, Anguita E, Hughes J, Sloane-Stanley JA, Sharpe JA, Koch CM, et al. Tissue-specific histone modification and transcription factor binding in {α} globin gene expression. Blood 2007;110:4503-10.
14. Flint J, Tufarelli C, Peden J, Clark K, Daniels RJ, Hardison R, et al. Comparative genome analysis delimits a chromosomal domain and identifies key regulatory elements in the α globin cluster. Hum Mol Genet 2001;10:371-82.
15. Sharpe JA, Summerhill RJ, Vyas P, Gourdon G, Higgs DR, Wood WG. Role of upstream DNase I hypersensitive sites in the regulation of human α globin gene expression. Blood 1993;82:1666-71.
16. Higgs DR, Wood WG, Jarman AP, Sharpe J, Lida J, Pretorius IM, et al. A major positive regulatory region located far upstream of the human α-globin gene locus. Genes Dev 1990; 4:1588-601.
17. Higgs DR, Sharpe JA, Wood WG. Understanding α globin gene expression: a step towards effective gene therapy. Semin Hematol 1998;35:93-104.
18. Sharpe JA, Chan-Thomas PS, Lida J, Ayyub H, Wood WG, Higgs DR. Analysis of the human α globin upstream regulatory element (HS-40) in transgenic mice. EMBO J 1992;11:4565-72.
19. Viprakasit V, Harteveld CL, Ayyub H, Stanley JS, Giordano PC, Wood WG, et al. A novel deletion causing α thalassemia clarifies the importance of the major human α globin regulatory element. Blood 2006;107:3811-2.
20. Higgs DR, Garrick D, Anguita E, De Gobbi M, Hughes J, Muers M, et al. Understanding α-globin gene regulation: aiming to improve the management of thalassemia. Ann N Y Acad Sci USA 2005;1054:92-102.
21. Tang XB, Feng DX, Di LJ, Huang Y, Fu XH, Liu G, et al. HS-48 alone has no enhancement role on the expression of human α-globin gene cluster. Blood Cells Mol Dis 2007;38:32-6.
22. Anguita E, Sharpe JA, Sloane-Stanley JA, Tufarelli C, Higgs DR, Wood WG. Deletion of the mouse α-globin regulatory element (HS-26) has an unexpectedly mild phenotype. Blood 2002;100:3450-6.
23. Vernimmen D, De Gobbi M, Sloane-Stanley JA, Wood WG, Higgs DR. Long-range chromosomal interactions regulate the timing of the transition between poised and active gene expression. EMBO J 2007;26:2041-51.
24. Anguita E, Hughes J, Heyworth C, Blobel GA, Wood WG, Higgs DR. Globin gene activation during haemopoiesis is driven by protein complexes nucleated by GATA-1 and GATA-2. EMBO J 2004;23:2841-52.
25. Waggoner SA, Liebhaber SA. Regulation of α-globin mRNA stability. Exp Biol Med 2003;228:387-95.
26. Aviv H, Voloch Z, Bastos R, Levy S. Biosynthesis and stability of globin mRNA in cultured erythroleukemic Friend cells. Cell 1976;8:495-503.
27. Clegg JB, Weatherall DJ, Milner PF. Haemoglobin Constant Spring: a chain termination mutant? Nature 1971;234:337-40.
28. Morales J, Russell JE, Liebhaber SA. Destabilization of human α-globin mRNA by translation anti-termination is controlled during erythroid differentiation and is paralleled by phased shortening of the poly(A) tail. J Biol Chem 1997;272:6607-13.
29. Kiledjian M, Wang X, Liebhaber SA. Identification of two KH domain proteins in the α-globin mRNP stability complex. EMBO J 1995;14:4357-64.
30. Weiss IM, Liebhaber SA. Erythroid cell-specific mRNA stability elements in the α 2-globin 3' nontranslated region. Mol Cell Biol 1995;15:2457-65.
31. Wang X, Kiledjian M, Weiss IM, Liebhaber SA. Detection and characterization of a 3' untranslated region ribonucleoprotein complex associated with human α-globin mRNA stability. Mol Cell Biol 1995;15:1769-77.
32. Weiss IM, Liebhaber SA. Erythroid cell-specific determinants of α-globin mRNA stability. Mol Cell Biol 1994;14:8123-32.
33. Ji X, Kong J, Liebhaber SA. In vivo association of the stability control protein αCP with actively translating mRNAs. Mol Cell Biol 2003;23:899-907.
34. Kong J, Liebhaber SA. A cell type-restricted mRNA surveillance pathway triggered by ribosome extension into the 3' untranslated region. Nat Struct Mol Biol 2007;14:670-6.
35. Schrier SL, Bunyaratvej A, Khuhapinant A, Fucharoen S, Aljurf M, Snyder LM, et al. The unusual pathobiology of hemoglobin constant spring red blood cells. Blood 1997;89:1762-9.
36. Liu H, Kiledjian M. An erythroid-enriched endoribonuclease (ErEN) involved in α-globin mRNA turnover. Protein Pept Lett 2007;14:131-6.
37. Ji X, Kong J, Carstens RP, Liebhaber SA. The 3' untranslated region complex involved in stabilization of human α-globin mRNA assembles in the nucleus and serves an independent role as a splice enhancer. Mol Cell Biol 2007;27:3290-302.
38. Komar AA, Kommer A, Krasheninnikov IA, Spirin AS. Cotranslational folding of globin. J Biol Chem 1997;272:10646-51.
39. Adachi K, Zhao Y, Surrey S. Effects of heme addition on formation of stable human globin chains and hemoglobin subunit assembly in a cell-free system. Arch Biochem Biophys 2003;413:99-106.
40. Adachi K, Zhao Y, Surrey S. Assembly of human hemoglobin (Hb) β- and γ-globin chains expressed in a cell-free system with α-globin chains to form Hb A and Hb F. J Biol Chem 2002;277:13415-20.
41. Shaeffer JR. Structure and synthesis of the unstable hemoglobin Sabin (α 2 β 2--91 Leu leads to pro). J Biol Chem 1973;248:7473-80.
42. Bargellesi A, Pontremoli S, Menini C, Conconi F. Kinetic evidence for the existence of α-globin pool in β-thalassemic reticulocytes. Eur J Biochem 1968;7:73-7.
43. Gill FM, Schwartz E. Free α-globin pool in human bone marrow. J Clin Invest 1973;52:3057-63.
44. Kihm AJ, Kong Y, Hong W, Russell JE, Rouda S, Adachi K, et al. An abundant erythroid protein that stabilizes free α-haemoglobin. Nature 2002;417:758-63.
45. Feng L, Gell DA, Zhou S, Gu L, Kong Y, Li J, et al. Molecular mechanism of AHSP-mediated stabilization of α-hemoglobin. Cell 2004; 119:629-40.
46. Zhou S, Olson JS, Fabian M, Weiss MJ, Gow AJ. Biochemical fates of α hemoglobin bound to α hemoglobin-stabilizing protein AHSP. J Biol Chem 2006; 281:32611-8.
47. Feng L, Zhou S, Gu L, Gell DA, Mackay JP, Weiss MJ, et al. Structure of oxidized α-haemoglobin bound to AHSP reveals a protective mechanism for haem. Nature 2005;435:697-701.
48. Weiss MJ, Zhou S, Feng L, Gell DA, Mackay JP, Shi Y, et al. Role of α-hemoglobin-stabilizing protein in normal erythropoiesis and β-thalassemia. Ann N Y Acad Sci 2005;1054:103-17.
49. Yu X, Kong Y, Dore LC, Abdulmalik O, Katein AM, Zhou S, et al. An erythroid chaperone that facilitates folding of α-globin subunits for hemoglobin synthesis. J Clin Invest 2007;117:1856-65.
50. Kong Y, Zhou S, Kihm AJ, Katein AM, Yu X, Gell DA, et al. Loss of α-hemoglobin-stabilizing protein impairs erythropoiesis and exacerbates β-thalassemia. J Clin Invest 2004;114:1457-66.
51. Viprakasit V, Tanphaichitr VS, Pung-Amritt P, Petrarat S, Suwantol L, Fishcer C, et al. Clinical phenotypes and molecular characterization of Hb H-Pakse disease. Haematologica 2002;87:117-25.
52. Turbpaiboon C, Limjindaporn T, Wongwiwat W, U-Pratya Y, Siritanaratkul N, Yenchitsomanus PT, et al. Impaired interaction of α-haemoglobin-stabilising protein with α-globin termination mutant in a yeast two-hybrid system. Br J Haematol 2006;-132:370-3.
53. Turbpaiboon C, Siritantikorn A, Thongnoppakhun W, Bundiworapoom D, Limwongse C, Yenchitsomanus PT, et al. Hemoglobin Pakse: presence on red blood cell membrane and detection by polymerase chain reaction-single-strand conformational polymorphism. Int J Hematol 2004;80:136-9.
54. Viprakasit V, Tanphaichitr VS, Chinchang W, Sangkla P, Weiss MJ, Higgs DR. Evaluation of α hemoglobin stabilizing protein (AHSP) as a genetic modifier in patients with β thalassemia. Blood 2004;103:3296-9.
55. Lai MI, Jiang J, Silver N, Best S, Menzel S, Mijovic A, et al. α-haemoglobin stabilising protein is a quantitative trait gene that modifies the phenotype of β-thalassaemia. Br J

©Ferrata Storti Foundation

Haematol 2006;133:675-82.
56. Dos Santos CO, Zhou S, Secolin R, Wang X, Cunha AF, Higgs DR, et al. Population analysis of the α hemoglobin stabilizing protein (AHSP) gene identifies sequence variants that alter expression and function. Am J Hematol 2008;83:103-8.
57. Galanello P, Perseu L, Giagu N, Sole G. AHSP expression in β-thalassemia carriers with thalassemia intermedia phenotype. Blood 2003;102:[abstract 1881].
58. Thein SL. Pathophysiology of {β} thalassemia: a guide to molecular therapies. Hematology (Am Soc Hematol Educ Program). 2005:31-7.
59. Kulozik AE, Kohne E, Kleihauer E. Thalassemia intermedia: compound heterozygous β0/β(+)-thalassemia and co-inherited heterozygous α(+)-thalassemia. Ann Hematol 1993; 66: 51-4.
60. Al Qaddoumi AA. Co-inheritance of α and β-thalassemia in a Jordanian family. Clin Lab Sci 2006;19:165-8.
61. Voon HP, Wardan H, Vadolas J. Co-inheritance of α- and β-thalassaemia in mice ameliorates thalassaemic phenotype. Blood Cells Mol Dis 2007;39: 184-8.
62. Steinberg MH. The interactions of α-thalassemia with hemoglobinopathies. Hematol Oncol Clin North Am 1991;5:453-73.
63. Rund D, Rachmilewitz E. Pathophysiology of α- and β-thalassemia: therapeutic implications. Semin Hematol 2001;38:343-9.
64. Sorensen S, Rubin E, Polster H, Mohandas N, Schrier S. The role of membrane skeletal-associated α-globin in the pathophysiology of β-thalassemia. Blood 1990;75:1333-6.
65. Schrier SL. Pathophysiology of the thalassemias. The Albion Walter Hewlett Award presentation. West J Med 1997;167:82-9.
66. Nagababu E, Fabry ME, Nagel RL, Rifkind JM. Heme degradation and oxidative stress in murine models for hemoglobinopathies: thalassemia, sickle cell disease and hemoglobin C disease. Blood Cell Mol Dis 2008;41: 60-6.
67. Nagababu E, Rifkind JM. Heme degradation by reactive oxygen species. Antioxidants Redox Signal 2004;6: 967-78.
68. Shinar E, Rachmilewitz EA. Oxidative denaturation of red blood cells in thalassemia. Semin Hematol 1990;27: 70-82.
69. Advani R, Rubin E, Mohandas N, Schrier SL. Oxidative red blood cell membrane injury in the pathophysiology of severe mouse β-thalassemia. Blood 1992;79:1064-7.
70. Yuan J, Rubin E, Aljurf M, Ma L, Schrier SL. Defective assembly of membrane proteins in erythroid precursors of β-thalassemic mice. Blood 1994;84:632-7.
71. Schrier SL, Centis F, Verneris M, Ma L, Angelucci E. The role of oxidant injury in the pathophysiology of human thalassemias. Redox Rep 2003;8:241-5.
72. Lutz HU, Fasler S, Stammler P, Bussolino F, Arese P. Naturally occurring anti-band 3 antibodies and complement in phagocytosis of oxidatively-stressed and in clearance of senescent red cells. Blood Cells 1988;14: 175-203.
73. Turrini F, Arese P, Yuan J, Low PS. Clustering of integral membrane proteins of the human erythrocyte membrane stimulates autologous IgG binding, complement deposition, and phagocytosis. J Biol Chem 1991;266: 23611-7.
74. Fucharoen S, Winichagoon P, Thonglairuam V, Wasi P. EF Bart's disease: interaction of the abnormal α- and β-globin genes. Eur J Haematol 1988; 40:75-8.
75. Cao A, Rosatelli C, Pirastu M, Galanello R. Thalassemias in Sardinia: molecular pathology, phenotype-genotype correlation, and prevention. Am J Pediatr Hematol Oncol 1991; 13:179-88.
76. Sanchaisuriya K, Fucharoen G, Sae-ung N, Sae-ue N, Baisungneon R, Jetsrisuparb A, et al. Molecular and hematological characterization of HbE heterozygote with α-thalassemia determinant. Southeast Asian J Trop Med Public Health 1997;28 (Suppl 3):100-3.
77. Wong WS, Chan AY, Yip SF, Ma ES. Thalassemia intermedia due to co-inheritance of β0/β(+)-thalassemia and (--SEA) α-thalassemia/Hb West-mead [α122(H5)His > Gln (α2)] in a Chinese family. Hemoglobin 2004;28: 151-6.
78. Fucharoen G, Trithipsombat J, Sirithawee S, Yamsri S, Changtrakul Y, Sanchaisuriya K, et al. Molecular and hematological profiles of hemoglobin EE disease with different forms of α-thalassemia. Ann Hematol 2006;85: 450-4.
79. Kanavakis E, Traeger-Synodinos J, Lafioniatis S, Lazaropoulou C, Liakopoulou T, Paleologos G, et al. A rare example that coinheritance of a severe form of β-thalassemia and α-thalassemia interact in a "synergistic" manner to balance the phenotype of classic thalassemic syndromes. Blood Cell Mol Dis 2004;32:319-24.
80. Wang C, Amato D. Chronic and severe haemolytic anaemia caused by co-inheritance of β-thalassaemia and triplicated α-globin genes. Br J Haematol 2007;137:489.
81. Premawardhena A, Fisher CA, Olivieri NF, de Silva S, Sloane-Stanley J, Wood WG, et al. A novel molecular basis for β thalassemia intermedia poses new questions about its pathophysiology. Blood 2005;106:3251-5.
82. Camaschella C, Kattamis AC, Petroni D, Roetto A, Sivera P, Sbaiz L, et al. Different hematological phenotypes caused by the interaction of triplicated α-globin genes and heterozygous β-thalassemia. Am J Hematol 1997; 55:83-8.
83. Traeger-Synodinos J, Kanavakis E, Vrettou C, Maragoudaki E, Michael T, Metaxotou-Mavromati A, et al. The triplicated α-globin gene locus in β-thalassaemia heterozygotes: clinical, haematological, biosynthetic and molecular studies. Br J Haematol 1996; 95:467-71.
84. Oron V, Filon D, Oppenheim A, Rund D. Severe thalassaemia intermedia caused by interaction of homozygosity for α-globin gene triplication with heterozygosity for β0-thalassaemia. Br J Haematol 1994;86:377-9.
85. Garewal G, Fearon CW, Warren TC, Marwaha N, Marwaha RK, Mahadik C, et al. The molecular basis of β thalassaemia in Punjabi and Maharashtran Indians includes a multilocus aetiology involving triplicated α-globin loci. Br J Haematol 1994; 86:372-6.
86. Sarakul O, Vattanaviboon P, Wilairat P, Fucharoen S, Abe Y, Muta K. Inhibition of α-globin gene expression by RNAi. Biochem Biophys Res Comm 2008;369:935-8.
87. Xie SY, Ren ZR, Zhang JZ, Guo XB, Wang QX, Wang S, et al. Restoration of the balanced α/β-globin gene expression in β654-thalassemia mice using combined RNAi and antisense RNA approach. Hum Mol Genet 2007;16:2616-25.
88. Voon HP, Wardan H, Vadolas J. siRNA-mediated reduction of {α}-globin results in phenotypic improvements in {β}-thalassemic cells. Haematologica 2008;93:1238-42.
89. Steensma DP, Gibbons RJ, Higgs DR. Acquired α-thalassemia in association with myelodysplastic syndrome and other hematologic malignancies. Blood 2005;105:443-52.
90. Gibbons RJ, Picketts DJ, Villard L, Higgs DR. Mutations in a putative global transcriptional regulator cause X-linked mental retardation with α-thalassemia (ATR-X syndrome). Cell 1995;80:837-45.

©Ferrata Storti Foundation