microRNA in erythrocytes

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Abstract

Mammalian erythrocytes are generally thought to lack RNA and therefore to be unable to translate new proteins in response to internal or external signals. Support for this long-standing view has accumulated from diverse studies, most of which have focused on the total content of RNA or the overall level of translation. However, more recent work on specific types of RNA has shown the presence in human erythrocytes of both Y RNA and microRNA. The latter seem particularly incongruous given that their normal role is to attenuate the translation of mRNA. Y RNA binds the Ro autoantigen which may have a role in cellular RNA quality control. Therefore the presence of both of these non-coding RNAs indicates the possible existence of other cryptic RNAs in erythrocytes. It also suggests either the existence of low levels of translation or new uncharacterized processes involving microRNA in these cells.

Erythrocyte biology

Vast numbers ($\sim 2–3 \times 10^{13}$) of erythrocytes circulate in the peripheral blood of an adult human and are essential for efficient gas exchange and transport throughout the body. Erythrocytes have evolved a highly specialized physiology, shape and structure for this crucial role: in the course of their development, mammalian erythroid cells eliminate most of the major structures normally present in eukaryotic cells, including nuclei, mitochondria, Golgi and endoplasmic reticulum. These changes are a vital part of a developmental programme that improves efficiency of gas exchange, increases the flexibility of the erythrocyte allowing easier passage through narrow vessels and reduces the effort required of the heart to pump the cells around the body [1]. However, the losses, particularly of the nucleus, also prevent erythrocytes from responding to internal and external cues by altering gene transcription and mRNA abundance. Once the nucleus is lost at the orthochromatic erythroblast–reticulocyte transition, no more RNA is synthesized, and any further regulation of gene expression is necessarily post-transcriptional.

In humans, reticulocytes are shed from bone marrow into the bloodstream and then over the next 1–2 days they mature to erythrocytes, during which time haemoglobin content increases, but both translational capacity and RNA content declines. Mature erythrocytes can be easily distinguished from their immediate reticulocyte progenitors by supravital staining with reagents such as New Methylene Blue. Reticulocytes exhibit pronounced staining of the eponymous reticulin which is thought to be a lattice of translating ribosomes in the final throes of haemoglobin production. Mature erythrocytes do not bind the dyes, hence a lack of detectable RNA is a defining feature of the mature erythrocyte. Microphotometry of mature erythrocytes after Azure B staining indicated no RNA present in mature rabbit erythrocytes [2], and measurements of RNA content during *in vitro* maturation of Friend leukaemia virus-transformed murine reticulocytes to erythrocytes showed the amount apparently asymptotically approaching zero by the end of the maturation period [3]. However, although mature rabbit reticulocytes indeed have diminished numbers of ribosomes compared with immature stages, a significant number were still readily observed by EM (electron microscopy) [4], suggesting that some may be retained in the mature erythrocyte stage that immediately follows. Larger structures such as mitochondria are clearly absent from erythrocytes, but the loss of these during erythropoiesis seems to be mechanistically unrelated to the loss of RNA [5]. For example, inhibitors of autophagy which prevent mitochondrial elimination do not affect the loss of RNA from developing reticulocytes [6]. The cell-wide RNA-destruction programme is probably carried out by ribonucleases such as that described from rabbit reticulocytes, which is active on rRNA, tRNA and probably mRNA [7,8]. However, it is not known how many ribonucleases participate or how selective they are in their substrate choice. Neither is it known whether all RNAs are degraded at equal or different rates or to the same or different extents. The meagre literature on this subject suggests that RNA disposal during erythropoiesis has received little attention, even though adequate destruction of RNA is thought to be important for red cell function (to recycle adenine nucleosides for ATP replenishment [9]).

miRNA (microRNA) in erythrocytes

Given the widely held view that erythrocytes have no significant RNA content, it comes as something of a surprise to find that erythrocytes maintain plentiful amounts of miRNA [10,11]. Both studies identified abundant miRNA

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Abbreviations used: EM, electron microscopy; miRNA, microRNA; miRNP, miRNA-containing ribonucleoprotein; RNP, ribonucleoprotein.
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in human erythrocytes, although only the latter excluded the possibility of contamination from other blood cells.

miRNAs are the best characterized class of several, novel, small (20–30 nt in length) RNAs that have been discovered within the last few years. Over 1000 different miRNAs are transcribed from the human genome and they exhibit widely varying patterns of expression and degrees of abundance. Their function is to regulate gene expression primarily by inhibiting the translation of mRNA [12,13], but translational promotion has also been reported [14]. Targeting the correct mRNA involves initial complementary Watson–Crick base-pairing between the mRNA and the miRNA. miRNAs accomplish this in partnership with a protein of the Argonaute family in an RNP (ribonucleoprotein) complex known as RISC (RNA-induced silencing complex) [15]. Thus the existence of miRNA in erythrocytes is surprising not only because of their presence, but also because the *raison d'être* of miRNA in other cells is to regulate gene expression through effects on mRNA translation, and this has long been thought to be absent from erythrocytes.

Other RNAs in erythrocytes

miRNAs are not, in fact, the first RNAs to have been found in erythrocytes. tRNA was reported to be present in mature erythrocytes [16], but the method by which reticulocytes were excluded as a possible alternative source of the RNA was not detailed. More convincingly, the small RNA polymerase III-transcribed transcripts Y1 and Y4 were shown to be present in mature erythrocytes [17]; density gradient depletion of reticulocytes from peripheral blood did not significantly diminish levels of these RNAs and neither were their levels altered much in blood samples exhibiting reticulocytosis. Therefore the authors concluded that significant amounts of the RNA must be present in mature erythrocytes. The absence of Y3 and Y5 RNA, which are normally present with Y1 and Y4 in nucleated cells, again illustrates how the loss of RNA during erythropoiesis is selective rather than wholesale and is more consistent with there being a reason for the maintenance of certain RNAs within erythrocytes than it being coincidence. Recent studies of Ro RNPs suggest that they function in the maintenance of integrity of other RNAs [18]. Therefore an obvious inference from the presence of Ro RNP in erythrocytes is that other RNAs exist in these cells whose integrity is important. The miRNAs described above are obvious candidates, but small amounts of rRNA and mRNA might benefit from protection and/or surveillance in the pro-oxidative environment of the erythrocyte.

Possible reasons for miRNA in erythrocytes

Chen et al. [10] showed that the miRNA pattern in human reticulocytes was very distinct from that in mature erythrocytes. Our own studies suggest the concentration of miRNA in erythrocytes is similar to that in nucleated cells and that the miRNAs are part of an active miRNP (miRNA-containing RNP) with Argonaute proteins (R. Lu and A.J. Hamilton, unpublished work). The selective retention of a distinct set of active miRNAs is more consistent with their having a function in erythrocytes than them being a random remnant of a general RNA-destruction programme. A direct role for the miRNA in the programmed destruction of RNA during erythropoiesis is unlikely because miRNA-directed cleavage of mRNA only occurs if near-perfect complementarity exists between miRNA and a mRNA [15] and very few human miRNAs have this property. Perhaps the most obvious explanation for the presence of miRNA in erythrocytes would be if these cells did, in fact, contain some translating mRNA upon which miRNPs could exert their acknowledged regulatory effects. However, this contradicts the well-established tenet that translation does not occur in these cells. There is good justification for the latter view. In countless routine clinical analyses on patient blood, erythrocytes fail to bind supravital dyes, a compelling testament to the drastic reduction in overall RNA content in these cells. It is also supported by studies of maturing reticulocytes showing a progressive reduction in RNA content to apparently zero levels [2,3,19]. As well as the evident reduction of RNA content, translational activity, as measured by uptake of radioactive amino acids, is close to zero in erythrocytes [20], and studies of reticulocytes show a decline in the ratio of polyribosomes to monosomes [4,21,22] with increasing maturity. However, the presence of miRNA, Y RNA and possibly tRNA show that the erythrocyte RNA content, although low, is not zero. It is possible then that the early studies might also have missed low levels of translation particularly if it were active only under certain conditions. Rifkind et al. [4], reporting an EM study of maturing reticulocytes, stated that there were no ribosomes in mature erythrocytes, but it is possible that, in such studies, a few ribosomes could have been overlooked owing to the electron density of haemoglobin. If mature erythrocytes have retained just a few select mRNA species, a few ribosomes might be enough to translate significant amounts of protein from these. With a low concentration of mRNA and ribosomes per cell, the relatively large amount of miRNA in erythrocytes could have a pronounced effect on mRNA expression. The dynamic engagement of miRNP with mRNA could provide the basis for a regulatory switch allowing erythrocytes greater responsiveness to internal and/or external cues.

It is also possible that the function of erythrocytic miRNPs is to regulate mRNA expression, but not in the erythrocyte. In this hypothesis, the large circulating erythrocytic pool would be the source of miRNPs that was transported and delivered to recipient cells in which they would reprogramme mRNA translation. The basis for this suggestion is recent reports that miRNA can be transported between cells in exosomes or microvesicles [23,24]. Erythrocytes produce microvesicles under certain disease or stress conditions [25], although it is not yet known whether these contain miRNP. Moreover, it remains to be determined whether miRNP in microvesicles can be taken up by cells in such a way

that they reprogramme mRNA expression in the recipient cell. However, it is an intriguing possibility, especially given the vast reservoir of erythrocytic miRNP that circulates in mammalian blood.

Conclusions

Despite textbook assertions to the contrary, mammalian erythrocytes do contain RNA. So far, only a few classes of RNA have been found to be present, suggesting highly selective retention during erythroid development. Further investigation is needed to fully characterize the erythrocyte 'RNome' and, from that, more insight into their function should follow. A reassessment of the translational status of erythrocytes specifically aimed at detecting low levels of translation would test one hypothesis, whereas a more detailed biochemical investigation of the erythrocytic miRNP would provide more clues to their function. In the meantime, none of the explanations for their presence is fully consistent with generally accepted views of erythrocyte biology. This suggests significant gaps in our understanding of erythrocytes. Filling these may well provide new insights into the functioning of these abundant and vital cells and offer prospects for novel therapeutic interventions.

References

1 Klinken, P.S. (2002) Red blood cells. Int. J. Biochem. Cell Biol. **34**, 1513–1518
2 Grasso, J.A., Woodard, J.W. and Swift, H. (1963) Cytochemical studies of nucleic acids and proteins in erythrocytic development. Proc. Natl. Acad. Sci. U.S.A. **50**, 134–140
3 Koury, M.J., Koury, S.T., Kopsombut, P. and Bondurant, M.C. (2005) *In vitro* maturation of nascent reticulocytes to erythrocytes. Blood **105**, 2168–2174
4 Rifkind, R.A., Danon, D. and Marks, P.A. (1964) Alterations in polyribosomes during erythroid cell maturation. J. Cell Biol. **22**, 599–611
5 Rapoport, S., Schmidt, J. and Prehn, S. (1985) Maturation of rabbit reticulocytes: susceptibility of mitochondria to ATP-dependent proteolysis is determined by the maturational state of reticulocyte. FEBS Lett. **183**, 370–374
6 Sandoval, H., Thiagarajan, P., Dasgupta, S.K., Schumacher, A., Prchal, J.T., Chen, M. and Wang, J. (2008) Essential role for Nix in autophagic maturation of erythroid cells. Nature **454**, 232–235
7 Burka, E.R. (1970) Ribonuclease activity in the reticulocyte cell-free system. Biochim. Biophys. Acta **209**, 139–144
8 Burka, E.R. (1969) RNase activity in erythroid cell lysates. J. Clin. Invest. **48**, 1724–1732
9 Kanno, H., Fujii, H. and Miwa, S. (2002) Physiological significance and molecular genetics of red cell enzymes involved in the ribonucleotide metabolism. Proc. Jpn. Acad. Ser. B Phys. Biol. Sci. **78**, 287–292
10 Chen, S.-Y., Wang, Y., Telen, M.J. and Chi, J.-T. (2008) The genomic analysis of erythrocyte microRNA expression in sickle cell diseases. PLoS ONE **3**, e2360
11 Rathjen, T., Nicol, C., McConkey, G. and Dalmay, T. (2006) Analysis of short RNAs in the malaria parasite and its red blood cell host. FEBS Lett. **580**, 5185–5188
12 Cannell, I.G., Kong, Y.W. and Bushell, M. (2008) How do microRNAs regulate gene expression? Biochem. Soc. Trans. **36**, 1224–1231
13 Ghildiyal, M. and Zamore, P.D. (2009) Small silencing RNAs: an expanding universe. Nat. Rev. Genet. **10**, 94–108
14 Vasudevan, S., Tong, Y. and Steitz, J.A. (2007) Switching from repression to activation: microRNAs can up-regulate translation. Science **318**, 1931–1934
15 Hutvagner, G. and Simard, M.J. (2007) Argonaute proteins: key players in RNA silencing. Nat. Rev. Mol. Cell Biol. **9**, 22–32
16 Merritt, J. and Desforges, J.F. (1963) Soluble RNA in adult red cell. Blood **22**, 836
17 Obrien, C.A. and Harley, J.B. (1990) A subset of Hy RNAs is associated with erythrocyte Ro ribonucleoproteins. EMBO J. **9**, 3683–3689
18 Sim, S., Weinberg, D.E., Fuchs, G., Choi, K., Chung, J. and Wolin, S.L. (2009) The subcellular distribution of an RNA quality control protein, the Ro autoantigen, is regulated by noncoding Y RNA binding. Mol. Biol. Cell **20**, 1555–1564
19 Burka, E.R. (1969) Characteristics of RNA degradation in the erythroid cell. J. Clin. Invest. **48**, 1266–1272
20 Holloway, B.W. and Ripley, S.H. (1952) Nucleic acid content of reticulocytes and its relation to uptake of radioactive leucine *in vitro*. J. Biol. Chem. **196**, 695–701
21 Warner, J.R., Rich, A. and Hall, C.E. (1962) Electron microscope studies of ribosomal clusters synthesizing hemoglobin. Science **138**, 1399–1403
22 Simpson, C.F. and Kling, J.M. (1968) The mechanism of mitochondrial extrusion from phenylhydrazine-induced reticulocytes in the circulating blood. J. Cell Biol. **36**, 103–109
23 Hunter, M.P., Ismail, N., Zhang, X., Aguda, B.D., Lee, E.J., Yu, L., Xiao, T., Schafer, J., Lee, M.-L. T., Schmittgen, T.D. et al. (2008) Detection of microRNA expression in human peripheral blood microvesicles. PLoS ONE **3**, e3694
24 Yuan, A., Farber, E.L., Rapoport, A.L., Tejada, D., Deniskin, R., Akhmedov, N.B. and Farber, D.B. (2009) Transfer of microRNAs by embryonic stem cell microvesicles. PLoS ONE **4**, e4722
25 Tibor, J.G. (2006) The how and why of exocytic vesicles. Transfusion **46**, 143–152

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