Micro-RNAs, New performers in multiple myeloma bone marrow microenvironment

Jahangir Abdi1,2, Lugui Qiu3 and Hong Chang1,2,3,4*

Abstract

The established interaction between multiple myeloma cells and bone marrow microenvironment components provides malignant cells with various survival, growth and drug resistance signals. As a new concept, identification of miRNAs and their related gene/protein targets, signaling molecules and pathways in the context of bone marrow microenvironment will help understanding more deeply the pathogenesis of the disease and possible mechanisms underlying environment-induced drug resistance. Recent studies suggest that bone marrow stromal cells can modulate some miRNAs (miR-21, miR-15a/16) in multiple myeloma cells through direct adhesion, cytokine secretion or transfer of miRNA-containing exosomes, however; the specific miRNA targets are not clear. In spite of a remarkable progress in understanding myeloma biology and therapy, the disease persists to be hard to treat. This review will discuss the most recent findings on miRNAs expression and function in the context of bone marrow microenvironment highlighting the miRNAs as potential therapeutic targets in multiple myeloma.

Keywords: Multiple myeloma, miRNA, Bone marrow stroma, CAMDR

Introduction

To survive and expand, multiple myeloma (MM) tumor cells have to be in a dynamic interaction with their stromal environment [1,2]. The profound effect of bone marrow stroma on malignant cells makes the bulk tumor resistant to drugs and invasive [2,3]. The latter interaction perhaps has been fully investigated in multiple myeloma. However, the not-clearly-determined mechanisms underlying cell adhesion-mediated drug resistance (CAM-DR) in MM cells in the context of bone marrow microenvironment (BMME) components warrants continuous research to allow identification of more potential drug targets. In developing drugs for MM treatment, little focus has been made on disrupting the above interaction, and variety of drug targets have been identified in isolated MM cells in no interaction with BMME [4-6]. Thus these systems cannot provide a more appropriate reflection of MM cells drug response in the bone marrow. miRNAs (miRs) have become an interesting research focus in MM as these molecules are mostly deregulated in MM, can have multiple targets (which may be oncogenes or tumor suppressor genes, e.g. MYC, TP53, PTEN) and have proved to significantly influence MM tumor in vitro and in vivo [7-12]. However, most findings cannot reflect the miRs biology in BMME context, while results obtained from some related B cells malignancies strongly support the concept that bone marrow stroma would have regulatory effects on some miR-mediated events including CAMDR.

miRNAs in MM: “small but stunning performers”

miRs belong to a class of small non-coding RNAs (22–25 nt. in length) which repress the expression of their target genes at translational level by binding to 3′UTR through partial sequence complementarity [13]. Recent studies indicate that miRs could play role in MM progression and pathobiology. It has been shown that the expression of some miRs is higher /or lower in MM cells compared to their normal counterparts [5,10,12]. miRs may target various genes involved in controlling cell cycle, proliferation and apoptosis. If the targets are tumor suppressor genes, miRs are considered as oncomiRs contributing to tumor pathogenesis, while some other miRs functioning as tumor suppressors could be downregulated by the oncogenic process. In support of this, Pichiorri et al. demonstrated that miRs 192, 194 and 215 in MM

* Correspondence: Hong.Chang@uhn.on.ca
1Division of Molecular and Cellular Biology, Toronto General Research Institute, Toronto, Canada
2Department of Laboratory Medicine & Pathobiology, University of Toronto, 172 St George St, Toronto, ON M5R 0A3, Canada
Full list of author information is available at the end of the article

© 2014 Abdi et al.; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
were positive regulators of p53 but repressed in newly diagnosed patients contributing to disease progression [8]. They also demonstrated that MGUS and MM displayed a unique miR expression signature [5]. For instance, miR-32 and miR-17~92 cluster were selectively upregulated in MM cells and HMCLs but not in monoclonal gamopathy of undetermined clinical significance (MGUS) and normal plasma cells. In addition, miRs19-a/b (part of the mir-17~92 cluster) downregulated suppressor of cytokine signaling-1 (SOCS-1) expression (which is normally an inhibitor of IL-6 signaling but frequently silenced in MM) thus maintaining MM cells growth and proliferation. Microarray analysis in another study also revealed a distinct mostly up-regulated miR signature in MM samples and cell lines compared to MGUS and normal plasma cells, with some miRs being associated with specific MM chromosomal isotypes [7]. The latter findings indicate that some miRs may play prognostic roles in MM. More importantly, miRs have also been indicated to mediate drug resistance in MM cells enforcing our understanding of the mechanism of chemoresistance in MM patients [14,15]. In spite of all the findings on miRs in MM with respect to involvement in various functional responses, the mechanism(s) regulating expression and function of miRs in MM has not been explicitly elucidated, but one possible mechanism may be epigenetic control of miR genes [16,17], however; our knowledge of this potential mechanism in MM is still scanty. Nonetheless, studies conducted in recent years on MM miR biology and function, have now opened a new window to MM targeted therapy exploiting miRNA analogues or their antagonists. More interestingly, combination of miRs or their inhibitors enhance anti-myeloma activity of chemotherapeutic drugs [18].

miR-mediated functions in MM cells in bone marrow microenvironment context

It is now understood that miRs mediate substantial functions in tumor microenvironment (reviewed at [19] (see Table 1). Bone marrow microenvironment (BMME) components especially bone marrow stromal cells and fibronectin protect MM cells against (drug-induced) apoptotic signals. This protective shield occurs mostly due to adhesion of MM cells to BMSCs leading to cytokine and growth factor secretion by later cells (or both), activation of various genes and signaling pathways in both cell types, and induction of cell adhesion mediated drug resistance (CAM-DR) in MM cells [20-22]. Regarding the fact that MM pathogenesis can largely be explained based on MM-stroma interactions, investigating miRs in this context will be more attractive in terms of detection of potential therapeutic targets. Notably, the role of miRs in modulating tumor cells interaction with stroma for maintaining tumor load, shaping metastasis and helping development of tumor-associated fibroblasts has been well highlighted [23,24].

Lin J et al. established a model comprising lymphoma cells adhered to BMSCs (HS-5 cell line) or lymph node stroma (HK cell line) [25,26]. Using global gene expression arrays, they found a remarkably changed expression pattern of several miRs in lymphoma cells adhered to stroma cells. More noticeably, miR-548m was underexpressed in adhered lymphoma cells but its ectopic overexpression suppressed stroma-induced clonogenic growth and drug resistance, and triggered apoptosis. Although very limited research as such has been performed in MM, obtaining closely related findings should not be unexpected. Xudong et al. studied the expression pattern and function of miR-21 in MM cells adhered to BMSCs and found that miR-21 expression was increased in HMCLs adhered to BMSCs which was partly regulated through NFκB signaling pathway [27]. They also observed that bortezomib reduced miR-21 expression in MM cell-BMSC co-culture, however; it was not clear whether bortezomib targeted MM cells, BMSCs or both to down-regulate miR-21.

In a more recent study, Emanuela et al. also demonstrated that HMCLs with low miR-21 expression (INA-6) displayed a high expression of miR-21 when adhered to BMSCs. miR-21 inhibition significantly decreased viability and clonogenic growth of MM cells but in stroma-free conditions miR-21 up-regulation did not affect proliferation or drug resistance. These findings are quite consistent with the results obtained in a previous study performed by Lin et al. In that study the same authors demonstrated that miR-21 over-expression in MM cells enhanced chemoresistance and impaired apoptosis in MM cells adhered to BMSCs [26].

Table 1 Expression and relevant regulatory functions of some investigated miRs in MM cells in the context of BMME components

| miRNA     | Expression pattern | Function                                                                 | Ref.               |
|-----------|--------------------|--------------------------------------------------------------------------|--------------------|
| miR-15a/16| Down-regulated     | Regulating MM cell proliferation through AKT3, MAPKs and NFκB pathways and by targeting cyclin D1, cyclin D2 and CDC25A | [10]               |
| miR-15a/16| Down-regulated     | Induction of BMSC-mediated drug resistance in MM cells, possibly through IL-6 | [28,35]            |
| miR-21    | Up-regulated       | Induction of BMSC-mediated drug resistance in MM cells partially through NFκB pathway and by targeting RhoB | [27]               |
| miR-199a-5p| Down-regulated   | Regulating bone marrow angiogenesis through targeting HIF-1α in MM cells and modulating interaction with BMSCs and bone marrow endothelial cells | [32]               |
| miR-135b  | Up-regulated       | Up-regulated in MM bone marrow mesenchymal stem cells following interaction with MM cells, impairing osteogenesis by targeting SMAD5 | [34]               |
| miR-199a, 24-3p, 15a-5p, 16-5p| Down-regulated | Down-regulated in MM mesenchymal cells, contributing to impaired osteogenesis | [33]               |
conditions [18]. Of note, they showed that anti-tumor activity in MM cell-BMSC co-cultures was induced only when MM cells were transfected with miR-21 inhibitor but not the BMSCs. This suggests that miR-21 inhibition in MM cells counteract protective function of BMSCs in co-culture experiments.

Roccaro et al. reported a miR signature in MM cells indicating downregulation of miR-15a/16-1 [10]. Specifically, they showed that miR-15a/16-1 ectopic expression reduced DNA synthesis, cell cycle and proliferation in MM cells, and decreased their adhesion to BMSCs. However, this study did not show the expression pattern and function of miR-15a/16-1 in MM cells following adhesion to BMSCs.

Later on, Hao et al. also observed that BMSCs maintained survival of MM cells and protected them from bortezomib-induced apoptosis through suppressing miR-15a in MM cells [28]. Of note, miR-15a/16-1 is located on chromosome 13q14 (a region most commonly deleted in MM), and complete absence of miR-15a/16-1 has been found in MM cells with this deletion [10]. On the other hand, another study revealed a heterogeneous expression pattern not correlated with chromosome 13 status [29]. Interestingly, it is reported that in B-chronic lymphocytic leukemia (B-CLL), miR-15a/16-1 are hosted by DLEU2, a regulatory gene which is frequently deleted in CLL leading to repression of miR-15-a/16 (thus cell cycle progression, proliferation and anti-apoptosis) [30]. It remains to be determined whether the above gene also hosts miR-15/16-1 in MM. BMSCs in MM could express miRs which should also be considered in MM pathogenesis. Reported expression of some miRs (miR-16, miR-223, miR-485-5p and miR-519d) and adhesion molecules genes in MM-BMSCs is higher than in normal counterparts [31]. Furthermore, some miRs have been reported to be modulated in BMSCs or MM bone marrow mesenchymal stem cells following interaction with MM cells contributing to angiogenesis induction (199a-5p) [32] or osteogenesis impairment (135b, 24-3b, 15a-5p) [33,34] (see also Table 1).

Mediators/targets of miR-related signaling in MM in the context of BMME

To understand the biology of miRs in MM cells in BMME, expression and function of miRs should be explored in above context, because only in that case can we trace any miRs link with bone marrow milieu-induced events especially CAM-DR. In fact since the effects of MM cells-BMSCs interaction are mutual, in experimental and animal models of MM-BMSC interaction it should be characterized what signals are triggering miRs expression alterations in MM cells (illustration at Figure 1). Does the BMSCs induce miR expression changes in MM cells through induction of adhesion-related signals in latter cells?, does it happen due to influence of some cytokines/mediators released by BMSCs?, is the altered expression of miRs in MM cell-BMSC co-cultures a function of co-operative interaction (miR change in both cell types) or the inductive effect of one cell type upon the other?, and finally, are miRs associated with integrin signaling pathways in MM cells following adhesion to ECM proteins such as FN? To find answers, several studies in recent years have provided some clues. For instance, it has been reported that IL-6 secreted by BMSCs increases drug resistance and reduces apoptosis of MM cells through suppression of miR-15a/16-1 [35], yet how IL-6 performs and what the miR targets are, have not been mechanistically characterized. More interestingly, another study showed that BMSCs transfer exosomes containing miR-15a into MM cells inducing their proliferation and survival [36]. Indeed miR-containing exosomes have been reported to play important roles in pathogenesis of various cancers [37]. RhoB, BTG, and PTEN have been indicated to be targets of miR-21 in MM cells [18], however; these findings were obtained in a stroma-free condition, thus not highlighting their real function in the context of bone marrow milieu. Lwin T et al. using in vitro and in vivo investigations found that stromal cells contributed to sustained c-Myc upregulation and miR-548m downregulation through a c-Myc/miR-548m feed-forward amplification loop leading to lymphoma cell growth and proliferation [26]. They also showed that miR-548m by directly targeting HDAC6, linked HDAC6 upregulation with lymphoma cell survival and drug resistance. Whether c-Myc can be similarly involved in miR-mediated functions in BMSC-MM cell interaction needs to be further investigated. The more important point in above study is the involvement of an epigenetic mechanism in controlling miR-induced responses. Indeed, epigenetic mechanisms have also been suggested to control miR-associated functional responses in MM cells [16]. Intriguingly, miRs 192, 194 and 215 (transcriptional targets of p53) were found to be hypermethylated in MM cells explaining their lower expression in MM than in MGUS [8,17]. However, it would be more interesting to investigate if BMSC or ECM triggers such regulatory mechanisms in MM cells, as given so, we may understand whether altered expression and function of some miRs following MM cell-BMSC/ECM interaction could underlie such events as CAM-DR. With our current knowledge, we still don’t know how miRs are modulated in MM cell-BMME context, and which critical oncopgenes or tumor suppressor genes are targeted by miRs in this context.

Concluding remarks and future prospects

Research on biology and function of miRs tends to achieve a hot spot in the field of MM therapy, with some evidence to introduce miRs as promising therapeutic
targets in MM [13]. However, most findings are indicative of MM cells drug responses in the absence of BMME irrespective of the fact that BMME plays a prominent role in the pathogenesis of MM. To this end, several in vitro and in vivo investigations have yielded important clues that some miRs (miR-21 and miR-15a/16-1) might play role in stroma-mediated drug resistance of MM cells but the specific targets and the underlying mechanisms are not clear yet. Moreover, the possibility of involvement of other miRs, their potential targets, and involved signaling pathways, warrants more in-depth research. As a matter of fact, reinforcing our knowledge of miRs expression and function in MM cell-BMME interaction will help us to find potential drug targets to overcome CAM-DR, which has been suggested to build the intrinsic (de novo) drug resistance and contribute to acquired drug resistance over time in MM patients [38-41].

Abbreviations
Akt: Also known as protein kinase B is a serine/threonine-specific protein kinase; BMSC: Bone marrow stromal cell; BTG: B cell translocation gene; CAM-DR: Cell adhesion-mediated drug resistance; ICAM-1: Intercellular cell adhesion molecule-1; JAK/STAT: Janus kinase/Signal transducer and activator of transcription; MAPK: mitogen activated protein kinase; MDM2: Minute double minute 2; mTOR: Mammalian target of Rapamycin; NFκB: Nuclear factor kappa-light-chain-enhancer of activated B cells; PI3K: Phosphatidylinositol-4,5-bisphosphate 3-kinase; PTEN: Phosphatase and tensin homolog; Ras: A member of small GTPase family; Rhod: Ras homolog gene family member B; VCAM-1: Vascular cell adhesion molecule-1; VLA4: Very late antigen 4.

Competing interest
The authors declare that they have no competing interest.

Authors’ contribution
JA participated in the design and drafted the review, LQ provided critical revision, HC designed, supervised the study, participated in drafting the review and provided critical revision; All authors read and approved the final manuscript.

Acknowledgement
The study was supported in part by the grants from Leukemia & Lymphoma Society of Canada, Cancer Research Society, open grant from national key lab for experimental hematology (project 2k-1102) and Gan-Po 555 project, Jiangxi, China, and also from Nature Science Foundation of China (project# 81172255).

Author details
1Division of Molecular and Cellular Biology, Toronto General Research Institute, Toronto, Canada. 2Department of Laboratory Medicine &
Pathobiology, University of Toronto, 172 St George St, Toronto, ON M5S 0A3, Canada. Institute of Hematology and Blood Disease Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China. Department of Laboratory Hematology, Laboratory Medicine Program, Toronto General Hospital, University Health Network, 200 Elizabeth Street, 11E-413, Toronto, Ontario M5G 2C4, Canada.

Received: 28 March 2014 Accepted: 6 May 2014
Published: 30 May 2014

References

1. Manier S, Sacco A, Leleu X, Ghobrial IM, Roccaro AM. Bone marrow microenvironment in multiple myeloma progression. J Biomed Biotechnol 2012; 2012:157496.

2. Gorgun GT, Whitehill G, Anderson JL, Hideshima T, Maguire C, Laubach J, Canada. 3Institute of Hematology and Blood Disease Hospital, Chinese Abdi et al. Biomarker Research China. 4Department of Laboratory Hematology, Laboratory Medicine Street, 11E-413, Toronto, Ontario M5G 2C4, Canada.

Deregulated microRNAs in multiple myeloma. J Biomed Biotechnol 2012, 2012:157496.

3. Tamura H, Ishibashi M, Yamashita T, Tanosako S, Okuyama N, Kondo A, Hyodo H, Shinya E, Takahashi H, Dong H, Tamada K, Chen L, Dan K, Oigata K. Marrow stromal cells induce B7-H1 expression on myeloma cells, generating an immunosuppressive microenvironment in multiple myeloma. Blood 2013, 121:2975–2987.

4. Vallet S, Podar K. New insights, recent advances, and current challenges targeting miR-21 inhibitors in vitro and in vivo multiple myeloma cell growth. Clin Cancer Res 2013, 19:2096–2106.

5. Sarron P, Kiess H. MicroRNAs in the tumour microenvironment: big role for small players. Endocr Relat Cancer 2013, 20:3925–3927.

6. Shain KH, Yadie DN, Meads MB, Huang M, Iove R, Hazlehurst LA. Dalton WS. Beta1 integrin adhesion enhances IL-6-mediated STAT3 signaling in myeloma cells: implications for microenvironment influence on tumor survival and proliferation. Cancer Res 2009, 69:1009–1015.

7. Gupta D, Tepon SP, Sharma Y, Hideshima T, Podar K, Tad K, Yin L, Lenzs S, Davies FE, Chauhan D, Schlossman RL, Richardson P, Ralph P, Wu L, Payandi, Muller G, Stirling D, Anderson KC. Adherence of multiple myeloma cells to bone marrow stromal cells upregulates vascular endothelial growth factor secretion: therapeutic applications. Leukemia 2001, 15:1950–1961.

8. Hideshima T, Mitsiades C, Tonon G, Richardson PG, Anderson KC. Understanding multiple myeloma pathogenesis in the bone marrow to identify new treatment targets. Nat Rev Cancer 2007, 7:585–598.

9. Li B, Kang Y. MicroRNAs as regulators of tumor-associated stromal cells. Oncotarget 2013, 4:2666–2676.

10. Zhang Y, Yang P, Wang XF. MicroRNA-mediated regulation of cancer metastasis by miRNAs. Trends Cell Biol 2014, 24:153–160.

11. Lin J, Liw T, Zhao J, Tam W, Choi YS, Moscinski LC, Dalton WS, Sotomayer EM, Wright KL, Tao J. Follicular dendritic cell-induced microRNA-mediated upregulation of PRDM1 and downregulation of BCL-6 in non-Hodgkin’s B-cell lymphomas. Leukemia 2011, 25:1452–1462.

12. Lwin T, Zhao X, Cheng F, Zhang X, Huang A, Shah B, Zhang Y, Moscinski LC, Choi YS, Koziolka AP, Bradford JE, Dalton WS, Sotomayer E, Tao J. A microenvironment-mediated c-Myc/miR-548/miR-15a/biochemical amplification loop in non-Hodgkin B cell lymphomas. J Clin Invest 2013, 123:4612–4620.

13. Wang X, Li C, Ju S, Wang Y, Wang H, Zhong R. Myeloma cell adhesion to bone marrow stromal cells confers drug resistance by microRNA-21 up-regulation. Leuk Lymphoma 2011, 52:1991–1998.

14. Hao M, Zhang L, An G, Meng H, Han Y, Xie Z, Xu Y, Li C, Yu Z, Chang H, Qi L. Bone marrow stromal cells protect myeloma cells from bortezomib-induced apoptosis by suppressing microRNA-15a expression. Leuk Lymphoma 2011, 52:1787–1794.

15. Cottrall SL, Jongen-Latourc M, De Knecht Y, Peeters JK, Bellerza LB, Lokhorst HM, Soneveld P, MicroRNA-15a and microRNA-16 expression and chromosome 13 deletions in multiple myeloma. Leuk Res 2010, 34:567–681.

16. Lerner M, Marada M, Loven J, Castro J, Davis Z, Oscier D, Henriksson M, Sangfelt O, Grander D, Henriksson M, Sonneveld P, Adrion KA, Dorcoran MM, DLEU2, frequently deleted in malignancy, functions as a host gene of the cell cycle inhibitory microRNA miR-15a and miR-16-1. Exp Cell Res 2009, 315:2941–2952.

17. Rimmah Berenstein BW, Axel N, Marlies W, Antonio P, Bernd D, Olga E. Aberrant expression of miRNA and miRNA of cell cycle and adhesion-related genes in bone marrow stromal cells derived from patients with multiple myelom. Blood 2013, 122:3149.

18. Lavinia Raimondi NA, Maria Teresa Di M, Emanuelia A, Maria Leotta DC, Annamaria A, Antonio Neri S, Taverna PDA, Riccardo A, Antonia Giordano P, Tassone TP. Targeting of multiple myeloma-related angiogenesis by miR-199a-5p mimics: in vitro and in vivo anti-tumor activity. Oncotarget 2014, 5.

19. Reigan YM MR, Yong Z, Patricia M, Salomon M, Yu-Tzu T, Priya D, Zhi Ning L, Masoumeh M, Antonio S, Yosra A, Susanna S, John R, Kaplan DL, Roccaro AM, Ghobrial IM. Microrna-dependent modulation of osteogenesis in a 3D in vitro bone marrow model system of multiple myeloma. Blood 2011, 122:3093.

20. Xu S, Cecilia Santini G, De Becker K, Vande Broek I, Leleu X, De Becker A, Van Camp B, Vanderkerken K, Van Riet I. Uregulation of miR-135b is involved in the impaired osteogenic differentiation of mesenchymal stem cells derived from multiple myeloma patients. PLoS One 2013, 8:e79752.

21. Hao M, Zhang L, An G, Sui W, Yu Z, Zou D, Xu Y, Chang H, Qi L. Suppressing miRNA-15a/16 expression by interleukin6 enhances drug-resistance in multiple myeloma cells. J Hematol Oncol 2011, 4:37.

22. Roccaro AM, Sacco A, Maio P, Azzaz A, Tai YT, Reigan A, Zhi Ning L, Moscinski LC, Antonio P, Bernd D, Olga E. Aberrant expression of miRNA and miRNA of cell cycle and adhesion-related genes in bone marrow stromal cells derived from patients with multiple myelom. Blood 2013, 122:3149.

23. Lavinia Raimondi NA, Maria Teresa Di M, Emanuelia A, Maria Leotta DC, Annamaria A, Antonio Neri S, Taverna PDA, Riccardo A, Antonia Giordano P, Tassone TP. Targeting of multiple myeloma-related angiogenesis by miR-199a-5p mimics: in vitro and in vivo anti-tumor activity. Oncotarget 2014, 5.

24. Reigan YM MR, Yong Z, Patricia M, Salomon M, Yu-Tzu T, Priya D, Zhi Ning L, Masoumeh M, Antonio S, Yosra A, Susanna S, John R, Kaplan DL, Roccaro AM, Ghobrial IM. Microrna-dependent modulation of osteogenesis in a 3D in vitro bone marrow model system of multiple myeloma. Blood 2011, 122:3093.
38. Meads MB, Hadlehurst LA, Dalton WS: The bone marrow microenvironment as a tumor sanctuary and contributor to drug resistance. Clin Cancer Res 2008, 14:2519–2526.
39. Shain KH, Dalton WS: Environmental-mediated drug resistance: a target for multiple myeloma therapy. Expert Rev Hematol 2009, 2:649–662.
40. Meads MB, Gatenby RA, Dalton WS: Environment-mediated drug resistance: a major contributor to minimal residual disease. Nat Rev Cancer 2009, 9:665–674.
41. Li ZW, Dalton WS: Tumor microenvironment and drug resistance in hematologic malignancies. Blood Rev 2006, 20:333–342.

Cite this article as: Abdi et al.: Micro-RNAs, New performers in multiple myeloma bone marrow microenvironment. Biomarker Research 2014 2:10.