[Frontiers in Bioscience, Scholar, 7, 217-225, June 1, 2015]

MicroRNA and ALK-positive anaplastic large cell lymphoma

Coralie Hoareau-Aveilla[1,2,3], Olaf Merkel[4,5], Fabienne Meggetto[1,2,3,4]

[1]Inserm, UMR1037 CRCT, F-31000 Toulouse, France, [2]Universite Toulouse III-Paul Sabatier, UMR1037 CRCT, F-31000 Toulouse, France, [3]CNRS, ERL5294 CRCT, F-31000 Toulouse, France, [4]European Research Initiative on ALK-related malignancies (ERIA), [5]Institute of Clinical Pathology, Medical University Vienna, 1090 Vienna, Austria

TABLE OF CONTENTS

1. ABSTRACT

In this review we describe the current literature covering the role of microRNA in anaplastic large cell lymphoma (ALCL). MicroRNA is one of the best characterized subgroups of non-coding RNAs and it is now becoming clear that its importance in oncogenesis has been greatly underestimated. In ALCL the deregulation of a diverse range of microRNA has been demonstrated however much less is known about the physiological consequences of this deregulation. Here we focus on the subgroup of ALCL bearing the anaplastic lymphoma kinase (ALK) translocation (ALK+). The pathways linking oncogenic ALK signaling and the regulation of microRNA are now becoming established with the transcription factor STAT3 appearing to play an important role in the epigenetic regulation. This review will discuss our current understanding of the role of microRNAs in ALK-mediated oncogenesis and will explain why we believe these new findings suggest that the use of methyltransferase inhibitors together with microRNA-specific drugs could be a useful addition to our current armamentarium in the fight against ALK(+) ALCL.

2. INTRODUCTION

MicroRNA are a class of small noncoding RNA that are around 22 nucleotides in length. To date, more than 2500 microRNA have been identified in humans (www.mirbase.org). MicroRNA act as post-transcriptional regulators of the expression of messenger RNA (mRNA). Their mode of action relies on their capacity to bind directly to their mRNA target via the RNA induced silencing complex (RISC) whose central protein is Ago2 in order to repress the translation process and/or to promote mRNA degradation. Since their discovery, microRNA have been associated with diverse physiological processes such as embryonic development, cell proliferation, cellular differentiation and apoptosis, among others (1, 2). Their expression is profoundly altered in many different types of cancer, including malignant hematopoiesis such as anaplastic large cell lymphoma (ALCL), a lymphoma with a T- or null-cell phenotype that is characterized by a decreased expression of the aβ-TCR heterodimer and CD3ε (1). The 2008 World Health Organization classification subdivided ALCLs into 2 groups distinguished by the presence or absence of anaplastic lymphoma kinase (ALK) expression: ALK-positive ALCL (an established entity) and ALK-negative ALCL (a proposed new entity) (3-7). Overall, the prognosis of ALK(+) ALCL is remarkably better than that of other T-cell lymphomas. The standard first-line of treatment for ALK(+) ALCL consists of doxorubicin-based polychemotherapy. This is associated with an overall response rate of 90 percent, a 5-year relapse-free survival rate of 60 percent and a 5-year overall survival rate of 70 percent.

ALK was first described in 1994 as part of the NPM/ALK fusion protein that is expressed in the majority of systemic anaplastic large cell lymphoma (ALCL). NPM/ALK is produced as a consequence of the chromosomal translocation t(2;5) that fuses the amino terminal region of NPM (nucleophosmin) to the entire intracytoplasmic domain of ALK. ALK(+) ALCL accounts for about 3 percent of adult non-Hodgkin's lymphoma and 10–15 percent of childhood lymphoma. The cell-transforming potential of ALK in tumors largely depends on its deregulated tyrosine kinase activity that results from spontaneous dimerization through the NPM amino terminal domain. Several downstream pathways are

Table 1. MicroRNAs as markers in NPM-ALK(+) anaplastic large cell lymphomas

MicroRNA	Expression	MicroRNA targets	References
miR-16	Downregulated	VEGF	(8)
miR-17-92	Overexpressed STAT3-dependent	BIM	(10, 13)
miR-21	Downregulated	DNMT1	(15)
miR-26a	Downregulated	iNOS	(35)
miR-29a	Downregulated STAT3-dependent Epigenetic silencing by DNMT1 and DNMT-3B	MCL1	(9)
miR-96	Downregulated	ALK	(16)
miR-101	Downregulated	mTOR	(12)
miR-135b	Overexpressed	GATA3, STAT6, FOXO1	(11)
miR-219	Downregulated	ICOS	(14)

STAT3: Signal transducer and activator of transcription 3; DNMT: DNA methyltransferase

activated by NPM/ALK with a broad range of signals that lead to increased cell proliferation, survival, motility, and cytoskeletal rearrangements. In ALCL, oncogenic ALK signaling is mediated by a series of key molecules and pathways, including STAT3, PI3K, Ras/MAPK/ERK, Shp2, p130Cas, PLCγ and Src (3, 7). It is likely that there are also other unidentified molecular pathways that interact with NPM/ALK to complement their effects. Several studies have highlighted the role of specific microRNA in oncogenic ALK signaling in ALK(+) ALCL (8-15) and even ALK itself has been proposed to be regulated by miR-96 (16) (Table 1). Thus, microRNA influence the cellular phenotype and pathogenesis of ALK(+) ALCL and are emerging as tissue-specific biomarkers with potential clinical applications for both identifying cancer subtypes and developing new therapies (18). In this way, an alternative strategy for preventing drug resistance in patients with ALK(+) ALCL will be to interfere with multiple ALK key pathways by targeting critical microRNA.

3. MICRORNA AND THE PATHOGENESIS OF ALK(+) ALCL

The deregulated expression of specific microRNA has been described in solid cancers, leukemias and lymphomas (2, 17-19). A limited number of studies also show different microRNA profiles in ALK(+) and ALK(-) ALCL (10, 12). We recently, performed the first comprehensive screen of deregulated microRNA expression downstream of ALK, using human formalin-fixed, paraffin-embedded (FFPE) tissue samples, ALCL cell lines and a CD4 transgenic ALK(+) ALCL tumor model. We identified that miR-101 is downregulated in all models (12). Moreover, the ectopic expression of miR-101 in ALCL cell lines reduced proliferation by increasing the number of cells arrested in G0/G1, and increased apoptosis in ALK(+) but not ALK(-) cell lines. These phenotypes likely result from the downregulation of miR-101 targets which include the anti-apoptotic protein Mcl-1 and the pro-survival serine/threonine kinase mTOR. To test the importance of the mTOR pathway in ALK-expressing tumors *in vivo*, mice were engrafted with ALK(+) ALCL tumors. (12). We observed a significant reduction in tumor growth when mice were treated with the mTOR inhibitor CCI-779 (temsirolimus), a rapamycin analogue. These data showed a dependence of ALK(+) cells on the mTOR pathway and suggest that the use of mTOR inhibitors could be a viable therapeutic strategy (12). In parallel, in the same study the authors also demonstrated that members of the miR-17–92 cluster (miR-17, miR-20a, miR-20b, miR-93, miR-106a) are overexpressed in ALK(+) ALCL compared with ALK(−) ALCL and, on the contrary, that miR-155 is expressed at a higher level in ALK(-) ALCL (12, 20). Expression of the miR-17–92 cluster in ALK(+) ALCL may reflect the pathogenesis of this disease because miR-17–92 microRNA have been associated with the inhibition of apoptosis, the promotion of proliferation, and the induction of tumor angiogenesis through repression of miR-17–92 targets such as E2F1 and the tumor suppressors PTEN and p21 (21, 22). Interestingly, a binding site for the transcription factor STAT3 has been identified in the promoter region of the miR-17–92 cluster (23), and a recent study showed that STAT3 is directly involved in the deregulation of the expression of the miR-17-92 cluster in ALK(+) ALCL (24). In contrast, the molecular mechanism leading to the repression of miR155 in ALK(+) ALCL is still unknown. In ALK(-) ALCL, the high miR-155 expression may be linked to a deregulated immune response and or may reflect the activated T-cell origin of ALCL. The reintroduction of miR-155 into NPM/ALK cell lines with low miR-155 levels (Karpas-299, SR786, SU-DHL-1 and SUP-M2) can actively downregulate its target protein, the CCAAT-enhancer-binding protein β (C/EBPβ), and enhance the expression of the inflammatory cytokine IL-22. High miR-155 led to a significantly enhanced tumor growth *in vivo* and increased IL-22 serum levels, suggesting that miR-155 is a tumor driver and is a potential therapeutic target in ALK(-) ALCL (20).

The small size of microRNA offers a unique advantage as an oncogenic biomarker since it is therefore less susceptible to enzymatic and mechanical degradation. In this way, Mehrotra and colleagues were the first to report miR-155 expression in FFPE sections of ALCL tumors through an *in situ* hybridization method (26). Importantly, this method allows the assessment of spatial microRNA expression patterns to

avoid the microRNA signature of any contaminating non-tumor cells that can affect the analysis of the microRNA profiling data. Our comprehensive screen of deregulated microRNA downstream of ALK also found that miR-146a is deregulated both in human ALCL cell lines and in the murine ALCL tumor model, suggesting that this microRNA may also be involved in the pathogenesis of ALK(+) ALCL (12). MiR-146a, like miR-155, is strongly induced by T-cell activation. A high expression of miR-146a in primary ALK(-) ALCL FFPE specimens was related to a low expression of its target protein, IL-1 receptor-associated kinase 1 (IRAK1), in ALK(-) cell lines. Moreover, miR-146a reintroduction into the ALK(+) cell lines Karpas-299 and SR786 reduced IRAK1 expression (20).

The pathognomonic features of ALK(+) ALCL can be determined readily by immunohistochemistry and the presence of the ALK gene rearrangement. We have identified a common set of deregulated microRNA that are present in different ALK(+) ALCL and which therefore may play important roles in the pathogenesis of ALK(+) ALCL. These findings corroborate previous data suggesting that ALK(+) and ALK(−) ALCLs are two distinct disease entities (3-5) and open the path to future research elucidating the specific roles of these microRNA in ALCL subtypes. One study by Liu and colleagues has already begun to look into this (10). They used genome-wide microRNA profiling of ALK(+) and ALK(-) ALCL to identify 7 deregulated microRNA: 5 were upregulated (miR-512-3p, miR-886-5p, miR-886-3p, miR-708, and miR-135b) and 2 were downregulated (miR-146a and miR-155) significantly associated with ALK(+) ALCL cases. The downregulation of miR-146a and miR-155 are already known to be signatures of ALK(+) ALCL cases and help validate these results. Interestingly, the upregulated microRNA define some important aspects of ALCL tumor biology, for example a high expression of miR-135b has also been shown to mediate the NPM-ALK–induced interleukin-17 (IL-17)-producing immunophenotype in ALK(+) ALCL (11). MiR-886 is known to target the pro-apoptotic BH3-only protein Bax, however whether miR-886 is a *bona fide* microRNA is currently under debate (25, 26). A high expression of miR-708 and miR-512-3p in ALK(+) ALCL may also be relevant since miR-708 has been shown to target transmembrane protein 88 (TMEM88), a negative regulator of Wnt signaling (27), and miR-512-3p targets the cell cycle inhibitor p21(Waf1/Cip1) (28). These two pathways have been previously shown to promote cell growth and tumorigenicity in ALK(+) ALCL (29, 30).

4. STAT3-MEDIATED MICRORNA GENE EXPRESSION IN ALK(+) ALCL

Signal transducer and activator of transcription 3 (STAT3) is a major downstream substrate for ALK and microRNA have been identified that are regulated by both NPM/ALK and STAT3 signaling. Thus, following ALK or STAT3 knock-down using inducible shRNA, Spaccarotella and colleagues performed genome-wide microRNA expression profiling in the ALK(+) ALCL cell line SUP-M2/TS. Forty-eight microRNA were found to be modulated in these ALK and STAT3 knock-down samples (13). To establish the role of deregulated microRNA in ALCL pathogenesis, they focused on the miR-17-92 cluster, a known oncogenic microRNA that has previously been demonstrated to be highly expressed in systemic ALK(+) ALCL (12). Pharmacological inhibition of STAT3, using the STAT3 inhibitor Stattic, decreased miR-17-92 cluster expression in all ALK(+) ALCL cell lines tested (SUP-M2/TS, JB-6, L82, and Karpas-299). In addition, the forced expression of the miR-17-92 cluster partially rescued the STAT3 knock-down by sustaining the proliferation and survival of NPM/ALK(+) cells both *in vitro* and in a xenograft mouse model. It also induced the downregulation of the pro-apoptotic protein BIM, suggesting that the miR-17-92 cluster might mediate resistance to STAT3 knock-down by targeting BIM. These data suggest that the miR-17-92 cluster could sustain the oncogenic properties of STAT3 in ALCL and that its inhibition might represent an alternative avenue to interfere with ALK signaling in ALCL. Thus, the authors suggested that the combination of a small molecule–based inhibitor of ALK with either a STAT3 inhibitor or an microRNA inhibitor may be useful to prevent chemoresistance in patients with ALK(+) ALCL (13). Other studies support this hypothesis. One such study, by Matsuyama and colleagues, used microarray analysis of microRNA expression to identify various signatures of aberrant microRNA expression in a wide range of hematologic cell lines, including T-cell lymphoma. In this analysis, ALCL were characterized by the increased expression of miR-135b, miR-21, and miR-27a, while miR-342, miR-454 and miR-324 were downregulated (31). Validation of these observations using quantitative RT-PCR analysis on 3 ALCL cell lines carrying the NPM-ALK fusion (Karpas-299, SUDHL-1, and SUP-M2) revealed that, among these microRNA, miR-135b was most prominently up-regulated in ALK(+) ALCL cell lines and its levels were reduced by the ALK inhibitor WHI-P154. In human primary ALK(+) ALCL samples, miR-135b levels were also elevated compared with ALK(-) ALCL samples. The NPM/ALK oncogene strongly promoted the expression of miR-135b and its host gene LEMD1 through activation of STAT3, as demonstrated by shRNA-mediated ALK or STAT3 knock-down (11). Inhibition of miR-135b suppressed the production of a Th17 proinflammatory cytokine, IL-17, by NPM/ALK cells. In accordance with the pro-angiogenic function of IL-17, miR-135b inhibition also reduced tumor angiogenesis and growth *in vivo,* demonstrating the significance of this microRNA as a potential therapeutic target (11). We have also demonstrated that NPM/ALK modulates the microenvironment (32), including the stimulation of angiogenesis, through a hypoxia-miR-16-dependent mechanism (8). Indeed, using our conditional NPM-ALK lymphoma transgenic mouse models (33, 34)

we observed that miR-16 was significantly downregulated in cells isolated from the lymph nodes of mice expressing the NPM-ALK transgene compared with cells isolated from mice in which the transgene was not induced (5). The overexpression of miR-16 inhibits the expression of the VEGF growth factor in ALK(+) ALCL cell lines and an inverse correlation between miR-16 and VEGF expression was observed in patient samples. Moreover, miR-16 injection into the tumors of nude mice was also found to decrease tumor growth *in vivo* (5).

MiR-21, miR-26a and miR-219 are also repressed by an NPM-ALK/STAT3-dependent mechanism and targeting them could represent another way to improve therapeutic approaches. The iNOS protein promotes lymphocyte survival and protects them from apoptosis. Whereas negligible levels of miR-26a were detected in ALK(+) ALCL cell lines (Karpas-299, DEL) and tumors, the expression of the iNOS protein was pronounced. Restoration of miR-26a was associated with a marked decrease in iNOS protein expression which was associated with a reduction in nitric oxide (NO) release from ALK(+) cells and a decrease in the viability, adhesion to endothelial cells and migration of these cells. The downregulation of NPM/ALK or STAT3 by siRNA was associated with a significant increase in miR-26a and a marked decrease in the iNOS protein (35).

5. NPM/ALK ACTS AS AN INDUCER OF EPIGENETIC SILENCING OF MICRORNA

Transcriptional silencing through DNA methylation of gene promoter and enhancer regions is a common epigenetic phenomenon in malignant cells (36). DNA methylation is an important epigenetic mechanism that silences genes transcribed by RNA polymerase II, including microRNA genes (37-41). It is directly mediated by three members of the DNA methyltransferase (DNMT) family, DNMT1, DNMT3A, and DNMT3B, which induce and maintain the methylation of CpG DNA dinucleotides. The dysregulation of microRNA can lead to aberrant DNA methylation in cancer. For instance, the miR-29 family (miR-29a, miR-29b and miR-29c) directly targets DNMT3A and DNMT3B. Similarly, DNMT1 is targeted by miR-148a, miR-152, miR-185 and miR-342 in several cancers (41, 42). DNA methylation can be reversed by treatment with DNMT inhibitors such as 5-azacytidine (5-aza-CR, Vidaza®) or the more stable 5-aza-2'-deoxycytidine (5-aza-CdR, decitabine, Dacogen®) (43, 44). In ALK(+) ALCL, DNA hypermethylation has been shown for several genes including those involved in T-cell receptor signaling and T-cell identity (15, 24, 45-50). High DNMT1 expression was also found in human ALCL cell lines and primary tumors (51). STAT3 is known to enhance the binding of DNMTs to promoters (52), and in ALK(+) ALCL cell lines STAT3 has been shown to functionally interact with DNMT proteins to promote promoter hypermethylation and gene silencing (53). In addition, STAT3 induces the transcription of the DNMT1 gene and favors DNMT1 expression by repressing miR 21, a microRNA shown to target the DNMT1 mRNA (15). Our laboratories showed for the first time that ALK(+) ALCL cell lines (SU-DH-L1, Karpas-299, Pio and Cost) and biopsy specimens express low levels of miR-29a (9). We found that the repression of miR-29a is dependent on NPM/ALK activity and STAT3, as demonstrated by siRNA-mediated STAT3 knockdown. This repression is likely mediated by the epigenetic silencing of miR-29a via DNMT1 and DNMT3b (7). This methylation was partially reversed by treatment with 5-aza-CdR and, albeit to a lesser extent, after NPM/ALK knockdown by siRNA. The DNA methylation was induced by NPM/ALK itself and probably via STAT3, as previously described (49, 50, 53). Moreover, DNMT3b, targeted by miR-29a, could amplify this mechanism in a positive feedback loop (54). Enforced miR-29a expression modulates apoptosis through inhibition of Mcl-1 expression in ALCL cell lines in a xenografted model, with a concomitant reduction in tumor growth. Decreased Mcl-1 expression correlated with an increased sensitivity of cells to doxorubicin (7). Thus, the activation of tumor-suppressive microRNA and the inhibition of oncogenic microRNA may have the potential to provide a fundamentally new approach for the development of therapeutics for many cancers, including ALK(+) ALCL. In conclusion, aberrant DNA methylation of microRNA genes is a potentially useful biomarker for detecting cancer and predicting its outcome.

6. CONCLUSIONS AND FUTURE DIRECTIONS

The replacement of tumor suppressive microRNA by lentiviral transduction or suppression of oncogenic microRNA using antisense strategies can be envisaged as novel approach to cancer therapy. The first clinical experiments using microRNA targeting antisense therapeutics have been performed in the context of hepatitis C and miR-122 (55). These treatments were effective and follow up studies have shown them to be safe (56), supporting the feasibility idea of using that this these type of drugs may be applicable also for other diseases. In particular, antisense therapeutics could target the miR-17-92 cluster in the case of ALK(+) ALCL and miR-155 in the case of ALK(-) ALCL. In murine experiments, delivery problems have been tackled by the direct injection of antisense drugs into the tumors themselves. Whether microencapsulation by nanoparticles and targeting of these by specific antibodies is feasible in humans remains to be seen (57). Another aspect that will be interesting to study is the presence of microRNA in vesicles released by the tumor cells (58). It has been shown that these vesicles are enriched for specific microRNA and thus could provide a means by which the tumor interacts with its environment.

Epigenetic silencing of miRNAs is involved in leukemogenesis, therefore the use of epigenetic drugs

able to induced re-expression of miRNAs alone or in combination with standard chemotherapy could provide a therapeutic advantage for resistant patients (59, 60). In that sense, de-methylating agents have already been used in myeloid malignancies with promising results (61, 62). Drug resistance is a major obstacle to the successful treatment of cancer. Although the majority of ALK(+) ALCL patients respond to polychemotherapy, the standard treatment for ALCL, new treatments are needed for resistant or relapsing patients (63-66). The use of crizotinib, an ALK tyrosine kinase inhibitor, has revolutionized the treatment of ALK(+) ALCL patients with advanced-stage or relapses (67, 68). However, acquired resistance inevitably occurs upon tyrosine kinase-targeted treatment strategies (68-70). The epigenetic silencing of microRNA genes is therefore critical for the oncogenic signaling driven by NPM-ALK. An interesting alternative strategy to overcome crizotinib resistance would be to use demethylating agents such as decitabine as adjuvant therapies.

7. ACKNOWLEDGEMENTS

This work was supported by grants from the INSERM, 'Fondation ARC pour la Recherche sur le Cancer', n° SFI20121205544 (FM) and the "Fondation de France n°Engt 201400047494 (FM)". CHA was under a contract supported the 'Fondation pour la Recherche Médicale'. English proofreading was performed by Scientific Scripts (http://scientificscripts.com). The present review was concerted inside the European Research Initiative of ALK-related malignancies (ERIA) (http://www.erialcl.net).

8. REFERENCES

1. D. Vasilatou, S. Papageorgiou, V. Pappa, E. Papageorgiou and J. Dervenoulas: The role of microRNAs in normal and malignant hematopoiesis. *Eur J Haematol*, 84(1), 1-16 (2010)
DOI: 10.1111/j.1600-0609.2009.01348.x

2. S. Parpart and X. W. Wang: microRNA Regulation and Its Consequences in Cancer. *Curr Pathobiol Rep*, 1(1), 71-79 (2013)
DOI: 10.1007/s40139-012-0002-7

3. A. J. Ferreri, S. Govi, S. A. Pileri and K. J. Savage: Anaplastic large cell lymphoma, ALK-positive. *Crit Rev Oncol Hematol*, 83(2), 293-302 (2012)
DOI: 10.1016/j.critrevonc.2012.02.005

4. A. J. Ferreri, S. Govi, S. A. Pileri and K. J. Savage: Anaplastic large cell lymphoma, ALK-negative. *Crit Rev Oncol Hematol*, 85(2), 206-15 (2013)
DOI: 10.1016/j.critrevonc.2012.06.004

5. F. B. Delsol G, Mûller-hermelink HK, Campo E, Jaffe ES, Gascoyne RD, Stein H, Kiney MC: Anaplastic large cell lymphoma, ALK-positive. IARC Press, Lyon (2008)

6. DY HN Mason, Delsol G, Stein H, Campo E, Kinney MC, Jaffe ES, Falini B: Anaplastic large cell lymphoma, ALK-negative. IARC Press, Lyon G (2008)

7. R. Chiarle, C. Voena, C. Ambrogio, R. Piva and G. Inghirami: The anaplastic lymphoma kinase in the pathogenesis of cancer. *Nat Rev Cancer*, 8(1), 11-23 (2008)
DOI: 10.1038/nrc2291

8. E. Dejean, M. H. Renalier, M. Foisseau, X. Agirre, N. Joseph, G. R. de Paiva, T. Al Saati, J. Soulier, C. Desjobert, L. Lamant, F. Prosper, D. W. Felsher, J. Cavaille, H. Prats, G. Delsol, S. Giuriato and F. Meggetto: Hypoxia-microRNA-16 downregulation induces VEGF expression in anaplastic lymphoma kinase (ALK)-positive anaplastic large-cell lymphomas. *Leukemia*, 25(12), 1882-90 (2011)
DOI: 10.1038/leu.2011.168

9. C. Desjobert, M. H. Renalier, J. Bergalet, E. Dejean, N. Joseph, A. Kruczynski, J. Soulier, E. Espinos, F. Meggetto, J. Cavaille, G. Delsol and L. Lamant: MiR-29a down-regulation in ALK-positive anaplastic large cell lymphomas contributes to apoptosis blockade through MCL-1 overexpression. *Blood*, 117(24), 6627-37 (2011)
DOI: 10.1182/blood-2010-09-301994

10. C. Liu, J. Iqbal, J. Teruya-Feldstein, Y. Shen, M. J. Dabrowska, K. Dybkaer, M. S. Lim, R. Piva, A. Barreca, E. Pellegrino, E. Spaccarotella, C. M. Lachel, C. Kucuk, C. S. Jiang, X. Hu, S. Bhagavathi, T. C. Greiner, D. D. Weisenburger, P. Aoun, S. L. Perkins, T. W. McKeithan, G. Inghirami and W. C. Chan: MicroRNA expression profiling identifies molecular signatures associated with anaplastic large cell lymphoma. *Blood*, 122(12), 2083-92 (2013)
DOI: 10.1182/blood-2012-08-447375

11. H. Matsuyama, H. I. Suzuki, H. Nishimori, M. Noguchi, T. Yao, N. Komatsu, H. Mano, K. Sugimoto and K. Miyazono: miR-135b mediates NPM-ALK-driven oncogenicity and renders IL-17-producing immunophenotype to anaplastic large cell lymphoma. *Blood*,

118(26), 6881-92 (2011)
DOI: 10.1182/blood-2011-05-354654

12. O. Merkel, F. Hamacher, D. Laimer, E. Sifft, Z. Trajanoski, M. Scheideler, G. Egger, M. R. Hassler, C. Thallinger, A. Schmatz, S. D. Turner, R. Greil and L. Kenner: Identification of differential and functionally active miRNAs in both anaplastic lymphoma kinase (ALK)+ and ALK- anaplastic large-cell lymphoma. *Proc Natl Acad Sci U S A*, 107(37), 16228-33 (2010)
DOI: 10.1073/pnas.1009719107

13. E. Spaccarotella, E. Pellegrino, M. Ferracin, C. Ferreri, G. Cuccuru, C. Liu, J. Iqbal, D. Cantarella, R. Taulli, P. Provero, F. Di Cunto, E. Medico, M. Negrini, W. C. Chan, G. Inghirami and R. Piva: STAT3-mediated activation of microRNA cluster 17~92 promotes proliferation and survival of ALK-positive anaplastic large cell lymphoma. *Haematologica*, 99(1), 116-24 (2014)
DOI: 10.3324/haematol.2013.088286

14. Q. Zhang, H. Wang, K. Kantekure, J. C. Paterson, X. Liu, A. Schaffer, C. Paulos, M. C. Milone, N. Odum, S. Turner, T. Marafioti and M. A. Wasik: Oncogenic tyrosine kinase NPM-ALK induces expression of the growth-promoting receptor ICOS. *Blood*, 118(11), 3062-71 (2011)
DOI: 10.1182/blood-2011-01-332916

15. Q. Zhang, H. Y. Wang, X. Liu, G. Bhutani, K. Kantekure and M. Wasik: IL-2R common gamma-chain is epigenetically silenced by nucleophosphin-anaplastic lymphoma kinase (NPM-ALK) and acts as a tumor suppressor by targeting NPM-ALK. *Proc Natl Acad Sci U S A*, 108(29), 11977-82 (2011)
DOI: 10.1073/pnas.1100319108

16. D. Vishwamitra, Y. Li, D. Wilson, R. Manshouri, C. V. Curry, B. Shi, X. M. Tang, A. M. Sheehan, Wistuba, II, P. Shi and H. M. Amin: MicroRNA 96 is a post-transcriptional suppressor of anaplastic lymphoma kinase expression. *Am J Pathol*, 180(5), 1772-80 (2012)
DOI: 10.1016/j.ajpath.2012.01.008

17. B. N. Davis-Dusenbery and A. Hata: MicroRNA in Cancer: The Involvement of Aberrant MicroRNA Biogenesis Regulatory Pathways. *Genes Cancer*, 1(11), 1100-14 (2010)
DOI: 10.1177/1947601910396213

18. G. De Tullio, V. De Fazio, N. Sgherza, C. Minoia, S. Serrati, F. Merchionne, G. Loseto, A. Iacobazzi, A. Rana, P. Petrillo, N. Silvestris, P. Iacopino and A. Guarini: Challenges and Opportunities of MicroRNAs in Lymphomas. *Molecules*, 19(9), 14723-14781 (2014)
DOI: 10.3390/molecules190914723

19. C. H. Lawrie: MicroRNAs in hematological malignancies. *Blood Rev*, 27(3), 143-54 (2013)
DOI: 10.1016/j.blre.2013.04.002

20. O. Merkel, G. Robert, F. Hamacher, L. Grabner, R. Greil and L. Kenner: Subtype specific expression of immnune-modulating miR-155 and miR-146a in anaplastic large cell lymphoma *Cancer Res*, 72(8), supplemental 1 (2009)

21. L. He, J. M. Thomson, M. T. Hemann, E. Hernando-Monge, D. Mu, S. Goodson, S. Powers, C. Cordon-Cardo, S. W. Lowe, G. J. Hannon and S. M. Hammond: A microRNA polycistron as a potential human oncogene. *Nature*, 435(7043), 828-33 (2005)
DOI: 10.1038/nature03552

22. J. T. Mendell: miRiad roles for the miR-17-92 cluster in development and disease. *Cell*, 133(2), 217-22 (2008)
DOI: 10.1016/j.cell.2008.04.001

23. M. Brock, M. Trenkmann, R. E. Gay, B. A. Michel, S. Gay, M. Fischler, S. Ulrich, R. Speich and L. C. Huber: Interleukin-6 modulates the expression of the bone morphogenic protein receptor type II through a novel STAT3-microRNA cluster 17/92 pathway. *Circ Res*, 104(10), 1184-91 (2009)
DOI: 10.1161/CIRCRESAHA.109.197491

24. C. Ambrogio, C. Martinengo, C. Voena, F. Tondat, L. Riera, P. F. di Celle, G. Inghirami and R. Chiarle: NPM-ALK oncogenic tyrosine kinase controls T-cell identity by transcriptional regulation and epigenetic silencing in lymphoma cells. *Cancer Res*, 69(22), 8611-9 (2009)
DOI: 10.1158/0008-5472.CAN-09-2655

25. H. Gal, G. Pandi, A. A. Kanner, Z. Ram, G. Lithwick-Yanai, N. Amariglio, G. Rechavi and D. Givol: MIR-451 and Imatinib mesylate inhibit tumor growth of Glioblastoma stem cells. *Biochem Biophys Res Commun*, 376(1), 86-90 (2008)
DOI: 10.1016/j.bbrc.2008.08.107

26. K. Lee, N. Kunkeaw, S. H. Jeon, I. Lee, B. H. Johnson, G. Y. Kang, J. Y. Bang, H. S. Park, C. Leelayuwat and Y. S. Lee: Precursor miR-886, a novel noncoding RNA repressed in cancer, associates with PKR and modulates its activity. *RNA*, 17(6), 1076-89 (2011) DOI: 10.1261/rna.2701111

27. J. S. Jang, H. S. Jeon, Z. Sun, M. C. Aubry, H. Tang, C. H. Park, F. Rakhshan, D. A. Schultz, C. P. Kolbert, R. Lupu, J. Y. Park, C. C. Harris, P. Yang and J. Jen: Increased miR-708 expression in NSCLC and its association with poor survival in lung adenocarcinoma from never smokers. *Clin Cancer Res*, 18(13), 3658-67 (2012) DOI: 10.1158/1078-0432.CCR-11-2857

28. V. Borgdorff, M. E. Lleonart, C. L. Bishop, D. Fessart, A. H. Bergin, M. G. Overhoff and D. H. Beach: Multiple microRNAs rescue from Ras-induced senescence by inhibiting p21(Waf1/Cip1). *Oncogene*, 29(15), 2262-71 (2010) DOI: 10.1038/onc.2009.497

29. S. A. Hegazy, A. Alshareef, P. Gelebart, M. Anand, H. Armanious, R. J. Ingham and R. Lai: Disheveled proteins promote cell growth and tumorigenicity in ALK-positive anaplastic large cell lymphoma. *Cell Signal*, 25(1), 295-307 (2013) DOI: 10.1016/j.cellsig.2012.09.027

30. A. M. Evens and R. B. Gartenhaus: Molecular etiology of mature T-cell non-Hodgkin's lymphomas. *Front Biosci*, 8, d156-75 (2003) DOI: 10.2741/922

31. C. H. Lawrie, N. J. Saunders, S. Soneji, S. Palazzo, H. M. Dunlop, C. D. Cooper, P. J. Brown, X. Troussard, H. Mossafa, T. Enver, F. Pezzella, J. Boultwood, J. S. Wainscoat and C. S. Hatton: MicroRNA expression in lymphocyte development and malignancy. *Leukemia*, 22(7), 1440-6 (2008) DOI: 10.1038/sj.leu.2405083

32. E. Dejean, M. Foisseau, F. Lagarrigue, L. Lamant, N. Prade, A. Marfak, G. Delsol, S. Giuriato, F. Gaits-Iacovoni and F. Meggetto: ALK+ALCLs induce cutaneous, HMGB-1-dependent IL-8/CXCL8 production by keratinocytes through NF-kappaB activation. *Blood*, 119(20), 4698-707 (2012) DOI: 10.1182/blood-2011-10-386011

33. S. Giuriato, N. Faumont, E. Bousquet, M. Foisseau, A. Bibonne, M. Moreau, T. Al Saati, D. W. Felsher, G. Delsol and F. Meggetto: Development of a conditional bioluminescent transplant model for TPM3-ALK-induced tumorigenesis as a tool to validate ALK-dependent cancer targeted therapy. *Cancer Biol Ther*, 6(8), 1318-23 (2007) DOI: 10.4161/cbt.6.8.4508

34. S. Giuriato, M. Foisseau, E. Dejean, D. W. Felsher, T. Al Saati, C. Demur, A. Ragab, A. Kruczynski, C. Schiff, G. Delsol and F. Meggetto: Conditional TPM3-ALK and NPM-ALK transgenic mice develop reversible ALK-positive early B-cell lymphoma/leukemia. *Blood*, 115(20), 4061-70 (2010) DOI: 10.1182/blood-2008-06-163386

35. H. Zhu, D. Vishwamitra, C. V. Curry, R. Manshouri, L. Diao, A. Khan and H. M. Amin: NPM-ALK up-regulates iNOS expression through a STAT3/microRNA-26a-dependent mechanism. *J Pathol*, 230(1), 82-94 (2013) DOI: 10.1002/path.4171

36. J. D. Best and N. Carey: Epigenetic opportunities and challenges in cancer. *Drug Discov Today*, 15(1-2), 65-70 (2010) DOI: 10.1016/j.drudis.2009.10.010

37. C. Baer, R. Claus and C. Plass: Genome-wide epigenetic regulation of miRNAs in cancer. *Cancer Res*, 73(2), 473-7 (2013) DOI: 10.1158/0008-5472.CAN-12-3731

38. M. Fabbri and G. A. Calin: Epigenetics and miRNAs in human cancer. *Adv Genet*, 70, 87-99 (2010) DOI: 10.1016/B978-0-12-380866-0.60004-6

39. L. Han, P. D. Witmer, E. Casey, D. Valle and S. Sukumar: DNA methylation regulates MicroRNA expression. *Cancer Biol Ther*, 6(8), 1284-8 (2007) DOI: 10.4161/cbt.6.8.4486

40. P. Lopez-Serra and M. Esteller: DNA methylation-associated silencing of tumor-suppressor microRNAs in cancer. *Oncogene*, 31(13), 1609-22 (2012) DOI: 10.1038/onc.2011.354

41. H. Suzuki, R. Maruyama, E. Yamamoto and M. Kai: DNA methylation and microRNA dysregulation in cancer. *Mol Oncol*, 6(6), 567-78 (2012) DOI: 10.1016/j.molonc.2012.07.007

42. H. Suzuki, R. Maruyama, E. Yamamoto and M. Kai: Epigenetic alteration and microRNA

dysregulation in cancer. *Front Genet*, 4, 258 (2013)
DOI: 10.3389/fgene.2013.00258

43. J. Goffin and E. Eisenhauer: DNA methyltransferase inhibitors-state of the art. *Ann Oncol*, 13(11), 1699-716 (2002)
DOI: 10.1093/annonc/mdf314

44. D. Subramaniam, R. Thombre, A. Dhar and S. Anant: DNA methyltransferases: a novel target for prevention and therapy. *Front Oncol*, 4, 80 (2014)
DOI: 10.3389/fonc.2014.00080

45. J. D. Khoury, G. Z. Rassidakis, L. J. Medeiros, H. M. Amin and R. Lai: Methylation of SHP1 gene and loss of SHP1 protein expression are frequent in systemic anaplastic large cell lymphoma. *Blood*, 104(5), 1580-1 (2004)
DOI: 10.1182/blood-2004-03-1151

46. T. Nagasawa, Q. Zhang, P. N. Raghunath, H. Y. Wong, M. El-Salem, A. Szallasi, M. Marzec, P. Gimotty, A. H. Rook, E. C. Vonderheid, N. Odum and M. A. Wasik: Multi-gene epigenetic silencing of tumor suppressor genes in T-cell lymphoma cells; delayed expression of the p16 protein upon reversal of the silencing. *Leuk Res*, 30(3), 303-12 (2006)
DOI: 10.1016/j.leukres.2005.08.012

47. R. Piazza, V. Magistroni, A. Mogavero, F. Andreoni, C. Ambrogio, R. Chiarle, L. Mologni, P. S. Bachmann, R. B. Lock, P. Collini, G. Pelosi and C. Gambacorti-Passerini: Epigenetic silencing of the proapoptotic gene BIM in anaplastic large cell lymphoma through an MeCP2/SIN3a deacetylating complex. *Neoplasia*, 15(5), 511-22 (2013)

48. Q. Zhang, H. Y. Wang, G. Bhutani, X. Liu, M. Paessler, J. W. Tobias, D. Baldwin, K. Swaminathan, M. C. Milone and M. A. Wasik: Lack of TNFalpha expression protects anaplastic lymphoma kinase-positive T-cell lymphoma (ALK+ TCL) cells from apoptosis. *Proc Natl Acad Sci U S A*, 106(37), 15843-8 (2009)
DOI: 10.1073/pnas.0907070106

49. Q. Zhang, H. Y. Wang, X. Liu and M. A. Wasik: STAT5A is epigenetically silenced by the tyrosine kinase NPM1-ALK and acts as a tumor suppressor by reciprocally inhibiting NPM1-ALK expression. *Nat Med*, 13(11), 1341-8 (2007)
DOI: 10.1038/nm1659

50. Q. Zhang, H. Y. Wang, M. Marzec, P. N. Raghunath, T. Nagasawa and M. A. Wasik: STAT3- and DNA methyltransferase 1-mediated epigenetic silencing of SHP-1 tyrosine phosphatase tumor suppressor gene in malignant T lymphocytes. *Proc Natl Acad Sci U S A*, 102(19), 6948-53 (2005)
DOI: 10.1073/pnas.0501959102

51. M. R. Hassler, A. Klisaroska, K. Kollmann, I. Steiner, M. Bilban, A. I. Schiefer, V. Sexl and G. Egger: Antineoplastic activity of the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine in anaplastic large cell lymphoma. *Biochimie*, 94(11), 2297-307 (2012)
DOI: 10.1016/j.biochi.2012.05.029

52. N. S. Thomas: The STAT3-DNMT1 connection. *JAKSTAT*, 1(4), 257-60 (2012)
DOI: 10.4161/jkst.22436

53. Q. Zhang, H. Y. Wang, A. Woetmann, P. N. Raghunath, N. Odum and M. A. Wasik: STAT3 induces transcription of the DNA methyltransferase 1 gene (DNMT1) in malignant T lymphocytes. *Blood*, 108(3), 1058-64 (2006)
DOI: 10.1182/blood-2005-08-007377

54. M. Fabbri, R. Garzon, A. Cimmino, Z. Liu, N. Zanesi, E. Callegari, S. Liu, H. Alder, S. Costinean, C. Fernandez-Cymering, S. Volinia, G. Guler, C. D. Morrison, K. K. Chan, G. Marcucci, G. A. Calin, K. Huebner and C. M. Croce: MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proc Natl Acad Sci U S A*, 104(40), 15805-10 (2007)
DOI: 10.1073/pnas.0707628104

55. H. L. Janssen, H. W. Reesink, E. J. Lawitz, S. Zeuzem, M. Rodriguez-Torres, K. Patel, A. J. van der Meer, A. K. Patick, A. Chen, Y. Zhou, R. Persson, B. D. King, S. Kauppinen, A. A. Levin and M. R. Hodges: Treatment of HCV infection by targeting microRNA. *N Engl J Med*, 368(18), 1685-94 (2013)
DOI: 10.1056/NEJMoa1209026

56. M. H. van der Ree, A. J. van der Meer, J. de Bruijne, R. Maan, A. van Vliet, T. M. Welzel, S. Zeuzem, E. J. Lawitz, M. Rodriguez-Torres, V. Kupcova, A. Wiercinska-Drapalo, M. R. Hodges, H. L. Janssen and H. W. Reesink: Long-term safety and efficacy of microRNA-targeted therapy in chronic hepatitis C

patients. *Antiviral Res*, 111C, 53-59 (2014) DOI: 10.1016/j.antiviral.2014.08.015

57. W. A. He, F. Calore, P. Londhe, A. Canella, D. C. Guttridge and C. M. Croce: Microvesicles containing miRNAs promote muscle cell death in cancer cachexia via TLR7. *Proc Natl Acad Sci U S A*, 111(12), 4525-9 (2014) DOI: 10.1073/pnas.1402714111
58. I. A. Babar, C. J. Cheng, C. J. Booth, X. Liang, J. B. Weidhaas, W. M. Saltzman and F. J. Slack: Nanoparticle-based therapy in an *in vivo* microRNA-155 (miR-155)-dependent mouse model of lymphoma. *Proc Natl Acad Sci U S A*, 109(26), E1695-704 (2012) DOI: 10.1073/pnas.1201516109
59. X. Agirre, J. A. Martinez-Climent, M. D. Odero and F. Prosper: Epigenetic regulation of miRNA genes in acute leukemia. *Leukemia*, 26(3), 395-403 (2012) DOI: 10.1038/leu.2011.344
60. J. Chen, O. Odenike and J. D. Rowley: Leukaemogenesis: more than mutant genes. *Nat Rev Cancer*, 10(1), 23-36 (2010) DOI: 10.1038/nrc2765
61. N. Amodio, M. Leotta, D. Bellizzi, M. T. Di Martino, P. D'Aquila, M. Lionetti, F. Fabiani, E. Leone, A. M. Gulla, G. Passarino, M. Caraglia, M. Negrini, A. Neri, A. Giordano, P. Tagliaferri and P. Tassone: DNA-demethylating and anti-tumor activity of synthetic miR-29b mimics in multiple myeloma. *Oncotarget*, 3(10), 1246-58 (2012)
62. W. Zhang, Y. E. Wang, Y. Zhang, X. Leleu, M. Reagan, Y. Zhang, Y. Mishima, S. Glavey, S. Manier, A. Sacco, B. Jiang, A. M. Roccaro and I. M. Ghobrial: Global Epigenetic Regulation of MicroRNAs in Multiple Myeloma. *PLoS One*, 9(10), e110973 (2014) DOI: 10.1371/journal.pone.0110973
63. K. V. Foyil and N. L. Bartlett: Brentuximab vedotin and crizotinib in anaplastic large-cell lymphoma. *Cancer J*, 18(5), 450-6 (2012) DOI: 10.1097/PPO.0b013e31826aef4a
64. B. Hallberg and R. H. Palmer: Mechanistic insight into ALK receptor tyrosine kinase in human cancer biology. *Nature Reviews Cancer*, 13(10), 685-700 (2013) DOI: 10.1038/nrc3580
65. A. Kruczynski, G. Delsol, C. Laurent, P. Brousset and L. Lamant: Anaplastic lymphoma kinase as a therapeutic target. *Expert Opinion on Therapeutic Targets*, 16(11), 1127-1138 (2012) DOI: 10.1517/14728222.2012.719498
66. C. Voena and R. Chiarle: The battle against ALK resistance: successes and setbacks. *Expert Opin Investig Drugs*, 21(12), 1751-4 (2012) DOI: 10.1517/13543784.2012.717930
67. Y. P. Mosse, M. S. Lim, S. D. Voss, K. Wilner, K. Ruffner, J. Laliberte, D. Rolland, F. M. Balis, J. M. Maris, B. J. Weigel, A. M. Ingle, C. Ahern, P. C. Adamson and S. M. Blaney: Safety and activity of crizotinib for paediatric patients with refractory solid tumours or anaplastic large-cell lymphoma: a Children's Oncology Group phase 1 consortium study. *Lancet Oncol*, 14(6), 472-80 (2013) DOI: 10.1016/S1470-2045(13)70095-0
68. C. Gambacorti Passerini, F. Farina, A. Stasia, S. Redaelli, M. Ceccon, L. Mologni, C. Messa, L. Guerra, G. Giudici, E. Sala, L. Mussolin, D. Deeren, M. H. King, M. Steurer, R. Ordemann, A. M. Cohen, M. Grube, L. Bernard, G. Chiriano, L. Antolini and R. Piazza: Crizotinib in advanced, chemoresistant anaplastic lymphoma kinase-positive lymphoma patients. *J Natl Cancer Inst*, 106(2), djt378 (2014) DOI: 10.1093/jnci/djt378
69. L. Mologni: Inhibitors of the anaplastic lymphoma kinase. *Expert Opin Investig Drugs*, 21(7), 985-94 (2012) DOI: 10.1517/13543784.2012.690031
70. D. Zdzalik, B. Dymek, P. Grygielewicz, P. Gunerka, A. Bujak, M. Lamparska-Przybysz, M. Wieczorek and K. Dzwonek: Activating mutations in ALK kinase domain confer resistance to structurally unrelated ALK inhibitors in NPM-ALK-positive anaplastic large-cell lymphoma. *J Cancer Res Clin Oncol*, 140(4), 589-98 (2014) DOI: 10.1007/s00432-014-1589-3

Key Words: ALK$^+$ ALCL, microRNA, STAT3, Review

Send correspondence to: Fabienne Meggetto, Department of Hematology and Immunology, UMR1037 Cancer Research Center of Toulouse (CRCT), Oncopole Toulouse, 31037 Toulouse Cedex 1, France, Tel: 33-5-274-1669, Fax: 33-5-8274-1685, E-mail: fabienne.meggetto@inserm.fr