WP1C Novel targets and treatments
miRNA workshop, Montpellier, October 12-13, 2012
Summary for external communication

Background
MicroRNAs (miRNAs) are endogenous non-coding 20-22 nucleotide long RNAs that can play an important regulatory role in human biology by targeting mRNAs complementary in sequence for cleavage or translational inhibition and repression. As DNA methylation and histone modification, miRNAs thereby serve to regulate gene expression without altering the underlying DNA sequence. As a result, the fine-tuning of gene expression reduces the quantity of the resulting specific cell protein or enzyme. These epigenetic mechanisms are involved in all kind of the cellular processes and play pivotal roles in e.g. cell development, differentiation, activation, proliferation and apoptosis.

MiRNAs have also been shown to have a central role in the development and regulation of the immune system, as well as in autoimmune disorders as RA, examples depicted in the figure below.

However, hundreds of different microRNAs are present in mammalian cells, and their number and effect can vary according to cell type and developmental stage. Although nowadays recognized as important regulatory mechanisms in health and disease, studies addressing their physiological roles and function are at an early stage and the complexity of cell and pathway regulations make close research collaborations in this field necessary to connect and apply the continuously generated knowledge.
Workshop opening by invited speakers
In October 2012 a miRNA workshop took place in Montpellier, France, to further elucidate the role of microRNAs in Rheumatoid Arthritis (RA) and other chronic inflammatory disease. BTCure and Adipoa commonly organized this workshop in order to update each other, align activities in this area and to identify potential collaborations and for comparison and future validation of achieved results.

The workshop was opened by external speakers (as Sylviane Muller and Reinhard Voll) giving an introduction to innovative approaches for the treatment of antibody-dependent autoimmune diseases. Examples of successful preclinical and clinical studies of other innovative approaches and autoimmune disease as SLE were presented: As the peptide P140 (Lupuzor™) shown to reduce T cell auto-reactivity, and interacting with the heat shock protein HSC70/hsp73 (and the auto-lysosomal chaperon activity involved in antigen degradation) and thus leading to reduced Ag presentation. Another example presented was the potential use of Bortezomib (a proteasome inhibitor currently applied for cancer indications) as a therapeutic agent against SLEs, since as shown in off label case studies with severely affected, unresponsive patients, to lead to complete remission in tested patients.

Moreover, general important features of immune-biologics developed for the treatment of chronic inflammatory disease were outlined, as bioavailability, specificity, weak toxicity, and stability, as the potential for multiple downstream effects.

An example for miRNA inhibitors, so called antagonirs (chemically modified and stable ssRNAs) was presented, as developed by the group of Markus Stoffel, who’s group is investigating antagonirs as potential tools to down-regulate of miRNA effects to treat disease as well as the potential use of miRNA itself as biomarkers in metabolic disease. The current challenge of targeting antagonirs and mirRNAs to the affected tissues was further briefly discussed.

So targeting questions are still among the issues to be solved to enable the potential use of miRNA-based biologic agents in RA. However, further efforts have to be taken to investigate if miRNAs may serve as a novel class of biomarkers for detection of early RA or different disease stages and types, and if they would represent clinical realistic diagnostic or prognostic tools.

BTCure presentations at a glance

MicroRNAs regulating the expansion and survival of proinflammatory T Helper cells by Mir-Farzin Mashrehi (Germany)
Currently, chronic inflammation cannot be cured through immunosuppression. Chronically reactivated memory effector T helper (Th) cells become resistant to immunosuppressive therapies and are found at the site of inflammation. Thus, the group in Berlin further investigated the pathogenic memory Th cell compartment as a target driving the chronic inflammation.

Assuming that Th cells involved in autoimmune inflammation have a history of repeated re-stimulation by persistent auto-antigens, they in vitro generated acutely (once) activated and chronically (four times) activated murine effector memory Th cells. From the miRNA transcriptomes of these cell populations, they have identified miRNAs differentially expressed between naive, once and repeatedly activated Th1, Th2 and Th17 cells, by high-throughput sequencing of miRNA expression libraries.
One of the identified miRNAs, namely miR-182, is highly induced upon antigenic stimulation of naïve Th cells in an IL-2 dependent manner. Mir-182 was shown to regulate the transcription factor Foxo1, a suppressor of proliferation in resting T cells. Specific inhibition of miR-182 in activated Th lymphocytes limited their clonal expansion in vitro and in vivo and thus ameliorated ovalbumin-induced arthritis in mice.

This group further highlighted another miRNA, and its potential function. In an extensive target search it was shown that effector and memory Th cell survival depends on the ratio of the apoptosis regulators Bim and Bcl-2. In chronically activated Th1 cells, this miRNA suppresses the pro-apoptotic gene Bim and therefore promotes their survival. Inhibition of this miRNA increases apoptosis in these cells in a Bim dependent manner. T-bet and Twist1 are necessary for antigen dependent induction of this miRNA in Th1 cells, and absence of Twist1 leads to Bim up-regulation. This leading to the thesis that, Twist1 induces the expression of this miRNA and therefore promotes the survival of in pathogenic Th1 cells at the site of inflammation. In T cells isolated from the inflamed tissue of patient suffering from Arthritis they found elevated expression of this miRNA.

The future plan of this group is to target the presented miRNAs in preclinical models of autoimmune diseases (RA, colitis and SLE) by systemic application of antagonirs (specific miRNA inhibitors) developed by Markus Stoffel and to evaluate their therapeutic value in depleting pathogenic T h cells in chronic inflammation.

Identification of a Novel MicroRNA-Gene Circuit in Human Lupus Nephritis? Evidence for Modulation of Kallikrein Genes by Mir-422 by Panagiotis Verginis (Greece)

As disease phenotype results from gene expression changes not only in the immune effector cells but also in the target organs, this BTCure group in Greece sought to identify novel genes within the kidney in Lupus Nephritis. RNA was isolated from renal biopsy samples of 12 subjects with proliferative or membranous lupus nephritis and 5 healthy controls. A 24-microRNA signature defines human lupus nephritis with 9 microRNAs up-regulated and 15 microRNAs down-regulated compared to normal tissue and mir-422a to exhibit the highest up-regulation relative to control tissues. Bioinformatics analysis predicted that mir-422a has a binding site in the 3'UTR of kallikrein 4 (KLK4) gene verified by HEK293 reporter gene assays. In order to monitor miR-422a/KLK4 expression during Lupus Nephritis progression, NZB/W F1 lupus prone mice were used. The up-regulation of miRNA-422a and down-regulation of KLK4 mRNA levels was shown to increase within the course of disease development in this model. These data implicate regulation of KLK4 by miR-422a as a key pathogenetic event in lupus nephritis and suggest a potentially protective role of these genes in immune-mediated renal disease.

miRNA in the pathogenic role of fibroblasts in RA and SSc. Steffen Gay (Switzerland)

Steffen Gay emphasized that miR expression needs to be seen also in other epigenetic regulatory mechanisms such as acetylation and methylation.

In this regard the IL-6 regulating miR 203 is not regulated by inflammatory cytokines, but by methylation. Special emphasis was given to the expression of the “Oncomir-1” cluster miR 17-92 which is induced by TNFa and thereby augmented by enhancing the NF-kB and p38 signalling in RASF as documented by reporter gene assays. Interesting is also that the passenger strand of certain miRNAs is highly functional, such as miR 34* which mediates apoptosis resistance in RASF (rheumatoid arthritis synovial fibroblasts) via regulating XIAP. The cooperation with Glasgow and Athens has also resulted in joint publications with respect to miR 155 and similarities between the response of SF to TNFa from mouse and men.

Comparing Synovial Fibroblast microRNA expression in mouse and men by Evangelos Ntougkos on behalf of Ioannis Pandis (Greece)

The group in Greece is investigating the interplay of immunocytes and resident cells and in particular, Synovial fibroblasts (SF) in RA that show reduced apoptosis, expression of adhesion molecules, unbalanced Matrix metalloproteinases (MMPs), and epigenetic modifications favouring sustenance.
The arthritis causing transgenic human TNFa mouse model, Tg197, exhibit a distinct miR expression profile. Validation assays confirmed the dysregulation of miR-223, miR-146a and miR-155 previously associated with human rheumatoid arthritis (RA) pathology. However, the Tg197 miR profile predicted previously unidentified microRNAs deregulated in RA patient’s SFs and indicates that miR-155 is not essential for TNF-driven arthritis.

Of the described miR-221/222 and miR-323-3p, the latter were also found significantly up-regulated in patient RASFs, suggesting for the first time their association with RA pathology. Custom in silico microRNA target functional analysis predicted the contribution of newly associated miRNAs to the deregulation of the Wnt/Cadherin signaling in RASFs. miR-323-3p was shown to activate Wnt pathway. WNT pathway activation may, in part, be mediated via BTRC down-regulation in RASFs.

The Tg197 mouse is a valid mouse model to study miRNA biology in SFs and possibly other key tissues/cell types in vitro and in vivo and can predict unidentified molecular perturbations relevant to human disease.

The further focus will be the role of mir221/222 in RA and investigation of antagonim treatment of Tg197 and KO of these miRs in the Tg197 SFs.

**miR-155 as a pro-inflammatory regulator of arthritis by Mariola Kurowska-Stolarska (UK)**

The UK group consists of well-known experts in the miRNA area. The group presented microRNA patterns in cells of the innate systems, as of monocytes, macrophages and dendritic cells.

Mariola Kurowska-Stolarska showed that miR-155 is upregulated in synovial membrane and synovial fluid macrophages from RA patients. The increased expression of miR-155 in synovial fluid CD14(+) cells was associated with lower expression of a miR-155 target: SHIP-1, an inhibitor of inflammation. Similarly, SHIP-1 expression was decreased in CD68(+) cells in the synovial lining layer in RA patients as compared with osteoarthritis patients. Overexpression of miR-155 in PB CD14(+) cells led to down-regulation of SHIP-1 and an increase in the production of pro-inflammatory cytokines. Conversely, inhibition of miR-155 in RA synovial CD14(+) cells reduced TNF-α production.

The UK group also showed a connection to the metabolic syndrome (MS), since RA is associated with increased prevalence of MS. It was highlighted however, that miRNA155 seems to have a different role in liver metabolism, and seemed to prevent over accumulation of lipids in the liver through its direct target LXR-alpha. So while miR-155 could be responsible for liver homeostasis by regulating Kupffer cells biology, it’s overexpression in other types of macrophages can lead to chronic inflammation (also recently shown to promote atherosclerosis via CCL2 in atherosclerotic plaque macrophages).

**Control of auto-immunity by micro RNAs by Stephan Blueml (Austria)**

Stephan Blueml’s presentation focused on the role of microRNA 155 in the development of arthritis in various models. While mice deficient in miR155 did not develop collagen-induced arthritis (CIA), they did show a pathogenic phenotype in serum transfer arthritis. Also, TNF-driven arthritis in hTNF-transgenic mice was similar in wt and miR155 deficient mice. This and work presented above show, that microRNA 155 is important in immunological events (autoimmunity, monocyte activation) that lead production of TNF but not in TNF effector functions.

**Stem cell therapies in osteoarticular disease: the Adipoa experience by Christian Jorgensen (France)**

Christian Jorgensen presented work on adipose-derived stem cells (ASCs) and showed first impressive results of their chondroprotective effect in OA upon injection. One of the miRNA examples given was miRNA 29a and its involvement in chondrogenesis. It was outlined that it would be important for future cell therapy to have biomarkers to follow up on SC distribution and phenotypes in vivo.
Other presentations of BTCure external speakers

- miRNA regulation of runx2 in osteogenesis. Valérie Geoffroy (Inserm France)
- miRNA-guided repression vs. biological robustness: revisiting the significance of miRNA-mediated regulation. Hervé Seitz (France)
- miRNAs and viruses. Monsef Benkirane (France)
- miRNA in human Thelper cell subsets. Hans Yssel (France)
- miRNAs and metabolic disorders. M. Stoffel (Switzerland): Latreille
- Involvement of the miRs 17-92 Cluster in inflammation. Philippe Georgel (France)

Follow up topics of the BTCure group

It was suggested to have a microRNA meeting annually and that the next workshop could be in Zurich, organized by Steffen Gay.

In the meantime the whole WP1C team plans to up-date each other in a Teleconference before Christmas or the IMI report is due Jan 25, organized by Christian Jörgensen (WP1C lead).

We have the following exemplary current or planned collaborations between BTCure partners

- Stephan Blueml and Mariola Kurowska-Stolarska, and potentially with Mir-Farzin Mashreghi
- The group of Evangelos Ntougkos is strongly collaborating with Prof. Gay’s group
- Panagiotis Verginis in open collaboration with Mir-Farzin Mashreghi (Radbruch group)

Partners were asked to outline in case there is a need for additional collaboration in the miRNA field with other partners in the consortium before the next meeting. Ongoing collaborations should result in common publications, acknowledging BTCure.

Generally open question and topics to be further discussed in the future by this group and/or to be communicated within the consortium

- Most economic way to obtain material, preform assays and experiments
- General EFPIA involvement, in particular
  - Feedback on investigated WP1C targets
  - Potential support from EFPIA partners on needed consumables, like DNA microarray platforms
- Support of functional analysis and validation of results through other –omics as proteomics and how the BTCure network and ongoing work of –omics groups could be aligned