These research projects, undertaken in partial fulfillment of the requirements for the MD degree at Sackler Faculty of Medicine, Tel Aviv University in 2013–2014, were considered the most outstanding of the graduating class

Detection of target genes of miR-125b

E. Ben Meir, L. Shaham and S. Izraeli

Functional Genomics and Childhood Leukemia Research, Cancer Research Center, Sheba Medical Center, Tel Hashomer, affiliated with Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel [eladbenmeir@gmail.com]

Background: Mir-125b is a highly evolutionary conserved microRNA (miRNA), encoded by two different loci on the human genome: miR-125b-1 on chromosome 11 and miR-125b-2 on chromosome 21. Mir-125b is highly expressed in hematopoitic stem cells (HSC) and enhances their survival. Over-expression of miR-125b in specific subtypes of myeloid and lymphoid leukemias provides resistance to apoptosis, enhances proliferation and blocks the differentiation of malignant cells. Each miRNA controls many cellular RNAs and proteins. Although a few target genes of miR-125b were already identified, the mechanism whereby this miR-125b exerts its pro-survival phenotype is still unknown.

Objectives: To identify unknown target genes of miR-125b.

Methods: Potential target genes of miR-125b were chosen by bioinformatics analysis of experimental data obtained in our lab, utilizing proteomics and gene expression of mouse lymphoid Ba/F3 cells transduced with miR-125b versus control empty vector. These data were further cross-examined with four publicly available bioinformatics analysis databases for predicted miRNA targets: Targetscan, Miranda, Microcosm and PicTar. Four potential target genes – *BBC3*, *MAPK14*, *CSNK2A1* and *CASP2* – were chosen for further analysis. To examine whether these chosen genes are directly targeted by miR-125b, we performed dual luciferase assay: human embryonic kidney (HEK) 293T cells over-expressing miR-125b, mutated miR-125b or empty vector were transfected with luciferase vector containing the 3`UTR of the mRNA of each potential target gene. The luciferase activity in the cells was then measured.

Results: The luciferase activities in the cells transfected with the vector containing the 3`UTR of two target genes, *BBC3* and *CASP2*, were significantly suppressed by 30–40% ($P < 0.01$) in the cells over-expressing miR-125b compared to the empty vector-expressing cells. These suppressions were abolished in the mutated miR-125b-expressing cells. These results indicate that these two genes are directly targeted by miR-125b.

Conclusions: The identification of two pro-apoptotic genes – *BBC3* and *CASP2* – as direct targets of miR-125b may explain the pro-survival phenotype of the cells expressing this microRNA.

Analysis of mechanisms of bacterial (*Serratia marcescens*) attachment and migration along fungal hyphae

T. Hover, J. Mitiagin and N. Osherov

Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel [talhover@gmail.com]

Background: Interactions between bacteria and fungi are well documented in the literature, including mutualism, commensalism and pathogenesis. An incidental finding in our laboratory showed the remarkable capacity of *Serratia marcescens* to migrate along the mycelium of Zygomycete molds, to the best of our knowledge a phenomenon not previously ascribed to this bacterium.

Objectives: We conducted a series of experiments to better define the nature of this phenomenon.

Methods: Two strains of *S. marcescens* were tested for their ability to migrate along the mycelia of several different fungi to test the specifity of the interaction. Bacterial migration over killed *Mucor mycelium* and over aerial hyphae of Mucor and Rhizopus was tested as well. The role of flagella, a filamentous appendage involved in bacterial locomotion, and fimbriae, hairlike appendages involved in adherence to surfaces, were tested using *S. marcescens* mutant strains.

Results: We found that migration of *S. marcescens* along fungal mycelium was not restricted to Zygomycete molds. It was noted, albeit slower, on several Basidiomycete spp. as well; interestingly, no migration was seen on any mold of the phylum Ascomycota. *S. marcescens* migration did not necessitate fungal viability or surrounding growth medium, as bacteria migrated along aerial hyphae as well. Flagellum-defective strains of *S. marcescens* were able to migrate the same distance as the wild-type, although significantly slower. Specific attachment, if it occurs, does not necessitate type 1 fimbrial adhesion since mutants defective in this adhesin migrated equally well or faster than the wild-type. Bacterial translocation along fungi is an active process, as it occurred on killed mycelia. At the molecular level, the migration mechanism seems to be multifactorial: flagella support, but are not a prerequisite for the process, and although bacterial migration probably necessitates some kind of specific interaction, suggested by the selectivity of the phenomenon, it is likely mediated by other means than type 1 fimbriae, a recognized gram-negative bacterial adhesin.

Conclusions: it has been postulated that bacterial virulence factors originally evolved to compete with eukaryotes in the environment.

A better understanding of the mechanisms allowing *S. marcescens* to attach to, and translocate along the mycelia of eukaryotic fungi may provide better ways to prevent and treat bacterial colonization of biotic and abiotic surfaces, thus decreasing the need for antibiotics.

Gene expression biomarkers to predict the outcome of immune-modulatory treatment in patients with relapsing-remitting multiple sclerosis

S. Anis, M. Gurevich, E. Hanael, P. Sonis and A. Achiron

Laboratory of Neurogenomics, Multiple Sclerosis Center, Sheba Medical Center, Tel Hashomer, affiliated with Sackler Faculty of Medicine, Tel Aviv University, Israel [saaranis@gmail.com]

Background: Several immune-modulatory treatments are known to be effective for relapsing-remitting multiple sclerosis (RRMS), in terms of reducing the number of relapses and the disease progression. However, each treatment is characterized by patients with an unsatisfactory response. Currently, there is no effective tool to predict treatment efficacy in the individual patient prior to administration.

Objectives: To evaluate peripheral blood predictive biomarkers for glatiramer acetate (GA) and interferon-beta (IFNβ) treatment outcome in RRMS patients.

Methods: We used 85 HG-U133A2 (Affymetrix) gene-expression microarrays to analyze peripheral blood samples taken prior to treatment initiation with GA [n=37, age 38.6 ± 10.3 years, disease duration 6.4 ± 1.2 years, expanded disability status scale (EDSS) 2.1 ± 0.2] and interferon-beta (IFNβ) (n=48, age 37.8 ± 10.5 years, disease duration 6.6 ± 1.1 years, EDSS 2.0 ± 0.2). Good treatment response at 2 years of treatment was defined as a reduction of at least one relapse compared with the 2 year rate prior to treatment combined with annual increase of up to 0.5 in the EDSS score. Statistical comparison of the baseline expression of differentiating genes between responders and non-responders was performed to identify potential markers for treatment response.

Results: Good clinical outcome was observed in 25/37 (67%) GA treated patients. A signature of 762 gene-transcripts differentiated between good and poor responders at baseline, significantly enriched with genes related to apoptosis ($P = 5.27E-05$) and inflammation ($P = 6.28E-05$). A three-gene classifier including *ACTR5*, *WDR45* and *PPP1R13B*, all known to be related to apoptosis, showed a robust discrimination rate for treatment response. Good clinical outcome was observed in 34 of 48 (70%) IFNβ-treated patients. A signature of 627 gene transcripts differentiated between good and poor responders. This signature was significantly enriched with genes related to T cell growth and proliferation ($P = 1.37E-03$) being over-expressed in good responders at baseline. A three-gene classifier including *PRUNE*, *POU6F1* and *TRD*, known to be related to cellular growth, showed a robust discrimination rate for treatment response.

Conclusions: Our results suggest that baseline gene expression biomarkers can predict treatment response for GA and IFNβ treatment, offering future clinical applications.