Identification of Novel Micrornas Associated with Epithelial-Mesenchymal Transition in Human Bladder Cancer by miRNA Chip Array Profiling

Introduction and Objective: It is well known that superficial bladder cancer (BC) and invasive BC have distinct biological characteristics. Epithelial-mesenchymal transition (EMT) has recently been implicated in invasive BC. But the exact mechanism of regulating EMT in BC is not well known. One possible regulator of EMT is microRNA (miRNA). Several miRNAs have been involved in the development of BC. In this study, we aimed to identify novel miRNAs which are associated with the invasiveness of EMT in BC.
Material and Methods: We used 3 non-invasive tumor derived BC cell lines (RT112, RT4 and DSH1), 6 advanced tumor derived BC cell lines (KU7, 253J, TCCSUP, T24, J82 and UMUC3). As a normal control, we used 1 immortalized (TERT-NHUC) and 1 normal human urothelial cells. We characterized BC cell lines by evaluating invasion ability and expression of EMT markers, namely E-cadherin, N-cadherin, vimentin and ZEB1. Protein expression was evaluated by immunoblotting. Invasion ability was evaluated with Matrigel Invasion Chamber. We performed comprehensive miRNA expression analysis of BC cell lines by miRNA chip array (TORAY 3D-Gene human miRNA oligo chip) probing totally 874 hsa-miRNAs and the results were validated by qPCR. We analyzed the correlation among miRNA expression cluster, EMT markers and invasion ability.
Results: The invasion ability was higher in the 6 cell lines derived from advanced cancer than the others from non-invasive cancer. E-cadherin was dominantly expressed in the 3 non-invasive cells. To the contrary, N-cadherin, vimentin and ZEB1 were dominantly expressed in the 6 invasive cells. An unsupervised hierarchical clustergram of miRNAs divided these cells into two major groups: the invasive BC cell lines and the others. Collectively, this miRNA major grouping was identical with the expression pattern of EMT markers and was correlated with the invasion ability. Indeed, we found that several miRNAs that are known to be EMT regulators were differentially expressed between the two groups. We also identified some novel miRNAs whose function in BC is still unknown.
Conclusions: We identified novel miRNAs which are related to invasion ability and EMT in bladder cancer. Further examination to determine the function of these candidate miRNAs is warranted.