MicroRNA profiling: novel perspectives on biology and therapy of Malignant Mesothelioma

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Malignant Mesothelioma is an asbestos-related aggressive cancer with a median survival limited to 12-18 months. Recent findings indicate that miRNA genes could function as potential oncogenes or tumour suppressor genes, contributing to cellular transformation and tumourigenesis. Here we report a microRNA profiling for human malignant mesothelioma in cultured cells and in tissue specimens. To compare miR expression profiles of different mesothelioma cells (MPP89 and REN) with human mesothelial cells (HMC-TERT), we exploited the microarray technology, by using LNA-modified oligonucleotide microarrays. We performed cross-evaluations of miRs expression by comparisons among the three different cell lines examined: HMC-TERT vs MPP89, HMC-TERT vs REN e MPP89 vs REN. We further validated these microarray data by Real-Time QRT-PCR using TaqMan probes. RNA was prepared by a standard procedure using Trizol (Invitrogen). In parallel, we assessed mRNA expression profile of the same cells by cDNA microarray. The 3,000 genes significantly expressed in mesothelioma cells were matched with the microRNAs by bio-informatics analysis using miRANDA, TargetScan, PicTar e Diana-microT. Furthermore, we analyzed miRs expression by Real-Time QRT-PCR in tissue sections from thirty mesothelioma specimens, representative of three different phenotypes (epithelioid, sarcomatoid and biphasic), as well as tissue sections of non tumoural mesothelium. Total RNA was isolated from fixed and paraffin-embedded tissues by the FFPE RNeasy Kit (Qiagen). Preliminary results indicate that several miRs are differentially expressed in mesothelioma cell lines versus mesothelial cells. In particular, we identified 34 miRNAs as overexpressed and 19 miRNAs as downregulated in tumours. In tumoral cells an higher number of miRNAs were upregulated in MPP89 (17) than in REN (8). The differential miRNA expression between MPP89 and REN may account for their distinct molecular and biological features. Interestingly, according to our previous data (Bertino P. et al., Thorax, 6: 690-5, 2007), MPP89 cells display a more aggressive phenotype, with higher chemoresistance. Real-Time QRT-PCR analysis on miR expression fold changes, confirmed the data obtained with microarrays. Expression of genes encoding signal proteins critical for mesothelioma and other cancers survival (e.g. tyrosine kinase receptors), were analyzed for association with miRNA expression patterns, in particular Kit (hsa-miR-181a), PDGFR(hsa-miR-17-5p, hsa-miR-106a and hsa-miR-143), HGF (hsa-miR-26a, hsa-miR-18a, hsa-miR-193b) ADAM9 (hsa-miR-17-5p, hsa-miR-106a, hsa-miR-30c, hsa-miR-30e) and BTG1 (hsa-miR-17-5p, hsa-miR-26a, hsa-miR-20a, hsa-miR-143). The analysis of tumour specimens also revealed a possible link with the histological types examined. These data altogether strongly suggest that several miRNAs may play a critical role in the onset and development of Malignant Mesothelioma and may be useful tools for the development of novel and highly specific therapeutic approaches for this malignancy.