Exosomal microRNA transfer varies with specific microRNAs functional in colorectal cancer and cellular differentiation

Marlies I. Moshammer1, Maria Kalipciyan1, Rupert Bartsch1, Günther G. Steger1, Roland Sedivy2,3, and Robert M. Mader1

1Department of Medicine I, Comprehensive Cancer Center of the Medical University of Vienna, Vienna, 2Department of Clinical Pathology, Landesklinikum/Clinical Center St. Pölten, St. Pölten, and 3Center of Pathology, Danube Private University, Krems/Donau Austria

Introduction

Exosomes are membranous microparticles (40 – 100 nm diameters) of endocytic origin that are released by a variety of cell types into the extracellular space [1]. These particles consist of a lipid bilayer membrane surrounding a small cytosol and contain various molecular constituents of their cell of origin, including proteins and nucleic acid material (all types of RNAs) [2]. MicroRNAs (miR), which are incorporated into exosomes as well, are short (19 – 24 nt) noncoding RNAs that play important roles in posttranscriptional gene silencing of target messenger RNAs [3]. It has been demonstrated that exosomes are “bioactive vesicles” as they promote intercellular communication and immunoregulatory processes by shuttling their containing molecules from one cell to another [4, 5]. In cancerous tissue, the expression of microRNAs and their exosomal transmission is often deregulated [6]. The aim of this study was to evaluate the incorporation of five functional microRNAs relevant in colorectal cancerous tissue due to their often dysregulated expression into exosomes (miR-10b-5p, miR-21-5p, miR-141-3p, miR-200c-3p and miR-375). Furthermore, we provide insight into exosomal microRNA transport in the context of cellular differentiation.

Materials and methods

Cell culture, exosome isolation, and RNA extraction

A cell culture model consisting of three colon adenocarcinoma cell lines was used: CaCo2, SW480, and HT29. These cell lines are distinguished by their genotype and degree of differentiation (G1, G2, and G3, respectively). Cells were grown in RPMI Medium 1640 + GlutaMAX™ (Gibco) supplemented with 10% FCS gold (PAA Laboratories, made exosome free via ultracentrifugation) and 25 mg/ml gentamycin (Gibco) at 37 °C in a 5% CO2 atmosphere. Exosomes were isolated by precipitation (ExoQuick-TC™, SBI). Total cellular and corresponding exosomal RNA was extracted using TRI Reagent® (Sigma) according to the manufacturer’s protocol.

Reverse transcription and real-time PCR quantification

cDNA was synthesized from total cellular and exosomal RNA using specific primers for miR-10b-5p, miR-21-5p, miR-141-3p, miR-200c-3p and miR-375 followed by real-time PCR using TaqMan Assays (Applied Biosystems). Results were referred to the house-keeping gene RNU48 as an internal standard using an efficiency-corrected ΔΔCt algorithm for calculations.
Results

Interestingly, the magnitude of incorporation of specific microRNAs relevant in colorectal cancer into exosomes seems to be individually regulated. In relation to the house-keeping gene RNU48, half of miR-141-3p was incorporated into exosomes, while only 1/6 of miR-200c-3p – although originating of the same microRNA-family – was transferred into exosomes (cellular microRNA level: 100%). Likewise, ~ 1/6 of cellular miR-10b-5p and miR-21-5p was detected in exosomes relative to RNU48. Even lower, figures were only 5% for miR-375. All microRNAs detected – with the exception of miR-375 – were lost by 1/4 up to 3/4 in exosomes with decreased cellular differentiation (CaCo2 compared with SW480 corresponding to G1 and G2, respectively). In contrast, miR-375 was lost with progressive dedifferentiation (SW480 compared with HT29). Most interestingly, miR-21-5p and miR-200c-3p were highly enriched in the exosomes of all three cell lines (reference: exosomal RNU48), whereas microRNA such as miR-375 showed little variation within the exosomal compartment. Others, although originating from the miR-200 family, where hardly transferred into exosomes (miR-141-3p). These data are illustrated for SW480, but were similar in the other investigated cell lines (Figure 1).

Conclusion

These results indicate that the process of microRNA incorporation is individually regulated for single microRNAs, even if they belong to the same superfamily. Some of them such as miR-21-5p or miR-200c-3p were highly enriched in exosomes. Moreover, cellular differentiation has a significant impact on the transfer of cellular microRNAs into exosomes.

Acknowledgment

This work was funded by NÖ Forschungs- und Bildungsges.m.b.H (NFB), project number LS10-021.

References