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mir-200c Regulates Induction of Apoptosis through CD95 by Targeting FAP-1

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SUMMARY

Tumor progression shares many characteristics with the process of epithelial-to-mesenchymal transition (EMT). Cells that have undergone an EMT are known to have an increased resistance to apoptosis. CD95/Fas is an apoptosis-inducing receptor expressed on many tissues and tumor cells. During tumor progression CD95 is frequently downregulated, and tumor cells lose apoptosis sensitivity. miR-200 microRNAs repress both the EMT-inducing ZEB1 and ZEB2 transcription factors. We now demonstrate that miR-200c sensitizes cells to apoptosis mediated by CD95. We have identified the apoptosis inhibitor FAP-1 as a target for miR-200c. FAP-1 was demonstrated to be responsible for the reduced sensitivity to CD95-mediated apoptosis in cells with inhibited miR-200. The identification of FAP-1 as an miR-200c target provides a molecular mechanism to explain both the downregulation of CD95 expression and the reduction in sensitivity of cells to CD95-mediated apoptosis that is observed in the context of reduced miR-200 expression during tumor progression.

INTRODUCTION

Micro(mi)RNAs are small, noncoding RNAs, 19–24 nucleotides in length, that negatively regulate expression of multiple genes either by inducing translational silencing or by causing degradation of the mRNA of the targeted gene (Bartel, 2009). It has been firmly established that miRNAs regulate many key cellular processes such as cell growth, differentiation, and death (Schickel et al., 2008). Recently, we and others identified the miR-200 family of miRNAs (miR-200a, 200b, 200c, 141, and 429) as both fundamental markers and powerful regulators of the process of epithelial-mesenchymal-transition (EMT) (Gregory et al., 2008; Park et al., 2008). miR-200 maintains the epithelial phenotype of tissues by suppressing expression of the EMT-inducing transcription factors ZEB1 and ZEB2 (Christoffersen et al., 2007; Gregory et al., 2008; Hurteau et al., 2007; Park et al., 2008). The repression of E-cadherin by ZEB1 and ZEB2 is characterized by a full-scale shift in phenotype indicative of EMT. A key step in the progression of carcinomas is the loss of an epithelial phenotype and the acquisition of mesenchymal characteristics in a manner highly reminiscent of EMT. The process of EMT is reversible, and a mesenchymal-epithelial-transition (MET) is found during metastases when tumor cells may become partially re-epithelialized (Savagner, 2001). Consistent with miR-200 being involved in the process of EMT and MET, it has been reported for a number of human cancers that, early during malignant transformation, members of the miR-200 family are downregulated but are subsequently upregulated in advanced stages of cancer (Peter, 2009).

Tumor progression is a multistep process during which cancer cells undergo a number of changes. These changes include acquisition of mutations in tumor suppressor pathways and in oncogenes (Hanahan and Weinberg, 2000). In addition, cells become more and more resistant to apoptosis, and as a result, refractory to therapy. One apoptosis pathway that is affected by these changes is the pathway activated by the death receptor, CD95 (Fas/APO-1). It is well established that cancer cells lose sensitivity to CD95-mediated apoptosis either through acquisition of mutations in CD95, downregulation of CD95, or upregulation of antiapoptotic proteins (Peter et al., 2005, 2007). CD95 induces apoptosis by forming a death-inducing signaling complex (DISC) at the receptor that contains FADD, caspase-8/10, and the caspase-8 regulator, c-FLIP (Peter and Krammer, 2003). An apoptosis inhibitor that affects CD95 signaling by regulating surface expression of CD95 is Fas-associated phosphatase-1 (FAP-1, also known as PTPN13, PTPL1, or PTP-BAS) (Sato et al., 1995). FAP-1 has been linked to resistance to apoptosis mediated by CD95 in many human cancers, and an inverse correlation between sensitivity to CD95-mediated apoptosis and the expression of FAP-1 is seen in several tumor types (Elnemr et al., 2001; Foehr et al., 2005; Ivanov et al., 2003; Lee et al., 1999, 2001; Li et al., 2000; Meinhold-Heerlein et al., 2001; Myc et al., 1999; Nakai et al., 2000; Ungefroren et al., 2001; Wieckowski et al., 2007).

We previously reported that among the 60 cell lines maintained by the National Cancer Institute (NCI60) cell lines representing early stages of cancer progression (type II tumor cells, which die through a mitochondria-dependent pathway) are more sensitive to CD95 ligand when compared to cell lines that have characteristics of later stages (type I tumor cells, which die without mitochondrial involvement). This provided a model for the changes in CD95 signaling that occur during tumor

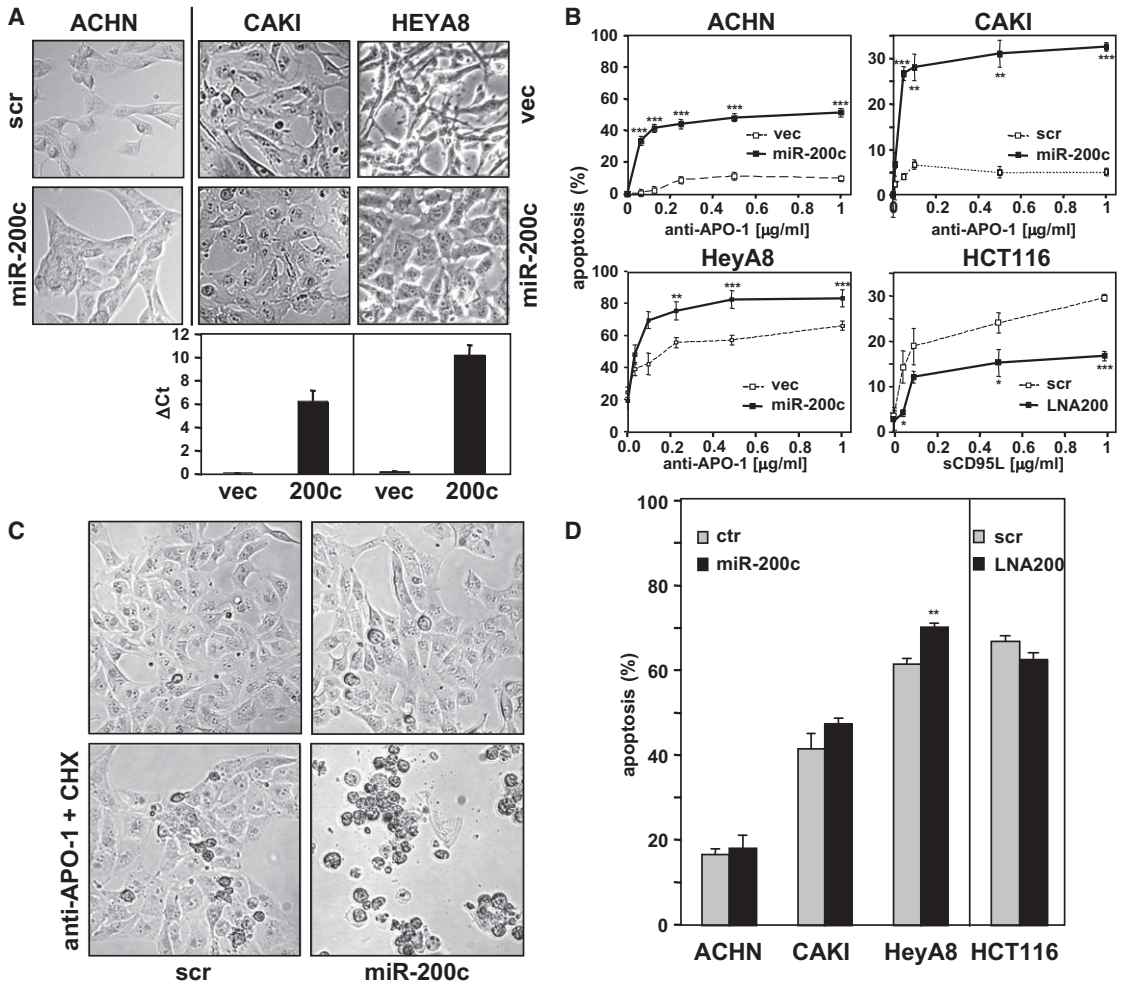

Figure 1. Altering Levels of miR-200 Changes Sensitivity of Cells to CD95-Mediated Apoptosis

(A) Phase contrast pictures showing morphological changes of cells transfected with scrambled pre-miRNA (scr) and pre-miR-200c (three times over 9 days) or cells stably infected with a retrovirus expressing scrambled oligo (vec) and miR-200c. Levels of mir-200c in retrovirus infected cells were quantified by real-time PCR.

(B) Cells shown in (A) stimulated through CD95. HCT116 cells were transfected with LNA200 seven times prior to stimulation. Cells ectopically expressing miR-200c or cells treated with LNA200 were stimulated with different concentrations of anti-APO-1 for 20 hr and apoptosis was quantified by MTS assay.

(C) CAKI-1 cells transfected as in (B) were treated with 1 μg/ml anti-APO-1 and 1 μg/ml cycloheximide for 20 hr. (D) Cells with increased or reduced levels of miR-200 were treated with 2 μg/ml of staurosporine for 20 hr and cell death was quantified by MTS assay. Asterisks indicate p values *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Note: different CD95 stimuli were used in the assays shown in (B) because type I cells are not sensitive to sCD95L, and type II cells are not sensitive to noncrosslinked anti-APO-1 (Algeciras-Schimnich et al., 2003). Values in graphs (A), (B), and (D) represent the mean ± SD from three independent experiments.

progression (Algeciras-Schimnich et al., 2003). We subsequently identified miR-200 to be more highly expressed in early/epithelial cancer cells (Park et al., 2008). We now report that miR-200 increases apoptosis sensitivity of cancer cells through targeting the apoptosis inhibitor, FAP-1. Our data provide a mechanistic explanation for how sensitivity of cancer cells to CD95-mediated apoptosis is lost early during neoplastic transformation.

RESULTS AND DISCUSSION

We previously noted that cells with properties of early cancers (type II cells) had an epithelial phenotype, whereas cells resembling more advanced cancer (type I cells) had a more mesenchymal phenotype (Algeciras-Schimnich et al., 2003). Because cancer cells lose apoptosis sensitivity during tumor progression including sensitivity to CD95-mediated apoptosis, we determined if changes in miR-200 levels affected CD95 signaling. miR-200c was introduced stably into CAKI-1 and HeyA8 cells, or transiently into ACHN cells. These three cell lines with different levels of sensitivity to CD95-mediated apoptosis possess mesenchymal features. Morphological changes consistent with acquisition of a more epithelial phenotype were noted in all three cell lines treated with miR-200c (Figure 1A). In addition, the miR-200c-treated cells showed increased sensitivity to