MicroRNAs (miRNAs) are small non-coding RNAs of 19-25 nucleotides that post-transcriptionally regulate the expression of target mRNA transcripts. They have been found involved in different processes commonly altered during tumorigenesis such as proliferation, differentiation and apoptosis. Here we will overview the last insights about microRNAs and apoptosis with the focus on two related miRs, miR-221&222, found involved in the resistance to TRAIL-inducing apoptosis and in the increased tumorigenesis of several types of cancers.

Introduction

Apoptosis, or programmed cell death, is a normal component of the development and health of multicellular organisms. Cells die in response to a variety of stimuli and during apoptosis they do so in a controlled, regulated fashion. In some cases the apoptotic stimuli comprise extrinsic signals such as the binding of death inducing ligands to cell surface receptors called death receptors (extrinsic apoptotic pathway). Death receptors belong to the tumor necrosis factor (TNF) superfamily that includes CD95 (FAS/APO-1), TNF-R1 and TRAIL receptors. All death receptors carry a conserved cytoplasmic domain of about 89 aa called the death domain (DD). The DD is structurally conserved protein-interaction domain (consisting of six antiparallel alpha-helices) which is important in the initiation of apoptotic signals. On ligand binding, death receptors trimerize and recruit adaptor molecules to form the death inducing signaling complex (DISC); this initiates a cascade of events leading to caspases activation and finally to cell death. In other cases apoptosis can be initiated by intrinsic signals that are produced following cellular stress (Intrinsic apoptotic pathway). Cellular stress may occur from exposure to radiation or chemicals or to viral infection. It might also be a consequence of growth factor deprivation or oxidative stress caused by free radicals. In general intrinsic signals initiate apoptosis via the involvement of the mitochondria and depend by the ratio of the various Bcl-2 proteins. MiRNAs are single-stranded RNAs (ssRNAs) of ~19-25 nt in length that are generated from endogenous hairpin-shaped transcripts [1] They play an important role in the negative regulation of gene expression by base-pairing to complementary sites on
the target mRNAs, usually in the 3′ untranslated region (UTR).

Binding of a miRNA to the target mRNA typically leads to translational repression and exonucleolytic mRNA decay, although highly complementary targets can be cleaved endonucleolytically. They have been found to regulate over 30% of mRNAs and have roles in fundamental processes, such as development, differentiation, cell proliferation, apoptosis and stress responses. The influence of regulatory noncoding RNA on apoptotic cell-signaling has not been extensively explored, but increasing evidence is pointing microRNAs as a controller of proliferative cell programs and apoptosis.

**Anti and pro-apoptotic miRs**

MicroRNA can act as anti and pro-apoptotic miRs, depending on the cellular contest and on the target genes they regulate. Among the proapoptotic miRNAs are miR-15 and miR-16, and the members of the miR-34 family. MiR-15a and miR-16-1 are deleted or downregulated in the majority of chronic lymphocytic leukemias (CLL) and their expression is inversely correlated to Bcl2 expression in CLL. BCL2 repression by these miRNAs induces the activation of the intrinsic apoptotic pathway in hematopoietic cancer cells. BCL2 and MYCN were identified as miR-34a targets and likely mediators of the tumor suppressor phenotypic effect in neuroblastoma [2]. The best characterized miRs with anti-apoptotic and tumorigenic function are miR-21, miR-155, miR-17-92, miR-106b-25, miR-221/222. Several studies indicate that miR-21 can act as an anti-apoptotic factor; in fact antisense inhibition of miR-21 caused significant apoptotic cell death in neuroepithelial cells through activation of caspases [3]. Ovcharenko and colleagues reported that the miR-155, among other microRNAs, affected negatively the activation of the caspase cascade [4]. That miR-106b-25 and miR-17-92 physiologically control apoptosis is supported by the work of Ventura and colleagues showing that miR-17-92/miR-106b-25 double knockout mice exhibit a
much more severe phenotype compared with miR-17-92 single knockout mice, characterized by prenatal lethality and extensive apoptosis in the liver, spinal cord, and lateral ganglionic eminences [5]. Recently, miR-221 and miR-222 have been shown to repress cyclin-dependent kinase (CDK) inhibitory proteins p27\textsuperscript{Kip1} and p57 as well as the c-Kit receptor, leading to cell proliferation and survival and inhibition of cell differentiation [6,7]. In a previous study, to identify mechanisms implicated in TRAIL resistance, we determined the microRNA expression profile in four NSCLC cell lines. We found that miR-221 and -222 are markedly up-regulated in TRAIL-resistant (Calu-1) and semi-resistant (A459, A549), versus TRAIL-sensitive NSCLC cells (H460). The results indicated that miR-221&222 modulate TRAIL sensitivity in lung cancer cells mainly by interfering with p27\textsuperscript{kip1} expression and TRAIL-induced caspase machinery [8]. In a more recent work we found that MET oncogene, through c-Jun transcriptional activation, up-regulates miR-221&222 expression which, in turn, by targeting PTEN and TIMP3, confers resistance to TRAIL-induced-cell death and enhances tumorigenicity of lung and liver cancer cells. The tumor suppressor PTEN regulates the PI3K/AKT pathway, a major cell survival pathway, playing a key role in the development of multiple drug resistance, including that to TRAIL. TIMP3, has been reported to induce the activation of both initiator caspases-8 and-9. The results suggest that therapeutic intervention, involving the use of these two microRNAs, should not only sensitize tumor cells to drug-inducing apoptosis but also inhibit their survival, proliferation and invasive capabilities [9]. Pineau and colleagues confirmed our results by profiling miRNA expression in 104 hepatocellular carcinoma (HCC) samples, 90 adjacent cirrhotic livers, 21 normal livers and 35 HCC cell lines. They found miR-221&222 as the most upregulated miRNAs in tumor samples, enhancing cell growth in vitro by targeting the CDK inhibitor p27/Kip1 and define disease progression from normal liver to full-blown tumors through liver cirrhosis. The tumor growth activity was efficiently inhibited by specific anti-miR-221&222. Finally they identified DNA damage-inducible transcript 4 (DDIT4), a modulator of mTor pathway, as a bona fide target of miR-221 [10].

**Conclusions**

Mounting evidence indicates that miRNA plays a fundamental role in tumorigenesis, controlling cell proliferation and apoptosis. Some miRNAs, such as miR-34 family, miR-15-16, have been shown to influence only the apoptotic pathway, whereas others, including miR-221&222, may play roles in both the apoptotic and cell-proliferation pathways (fig. 1). MiR-221&222 are among the most deregulated miRNAs implicated in cancer [8]. Their expression is highly upregulated in a variety of solid tumors, including thyroid cancer, hepatocarcinoma
and melanoma cells. Elevated miR-221&222 expression has been causally linked to proliferation, apoptosis and migration of several cancer cell lines. In this contest, in the near future, it will be possible to use these two microRNAs or compounds interacting with those and other miRNAs, as new therapeutic agents to induce apoptosis in cancer patients.

References


Authors

Michela Garofalo, PhD, Ohio State University, Prof. Gerolama Condorelli, University of Naples (corresponding author)

Contact

University of Naples Federico II - Department of Cellular and Molecular Biology and Pathology
via Pansini 5
80131 Naples
Italy