MINI-REVIEW

miR-421, miR-155 and miR-650: Emerging Trends of Regulation of Cancer and Apoptosis

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Abstract

It is becoming progressively more understandable that between transcription and translation there lies another versatile regulator that quantitatively controls the expression of mRNAs. Identification of miRNAs as key regulators of wide ranging signaling cascades and modulators of different cell-type and context dependent activities attracted basic and clinical scientists to study modes and mechanisms in details. In line with this approach overwhelmingly increasing *in vivo* and *in vitro* studies are deepening our understanding regarding miR-421, mir-155 and miR-650 mediated regulation of cellular activities. We also attempt to provide an overview of long non coding RNAs.

Keywords: miRNA - cancer - apoptosis - signaling

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Introduction

MicroRNAs have emerged as multifunctional regulators of wide ranging cellular activities. miRNAs are further categorized into tumor suppressor, cancer promoting (oncomirs) and metastasis promoting (metastamirs). miRNA biology is a well orchestrated mechanism that occurs both in nucleus and cytoplasm. RNA polymerase II or III mediate transcription of pri-miRNA. It is cleaved in the nucleus by the microprocessor complex (consisting of the RNase III endonuclease Drosha and the co-factor DGCR8. Recently it has been reported that knockdown of DGCR8 in primary fibroblasts induced senescent phenotype (Gómez-Cabello et al., 2013). Yet another contemporary study revealed that targeted inhibition of DGCR8 remarkably reduced migratory and invasive potential of ovarian cancer cells (Guo et al., 2013). There is another exciting piece of information highlighting role of post-translational modifications for protein stability of Drosha. Drosha is degraded via ubiquitination however if the residues are acetylated, Drosha escapes from degradation. It has been experimentally verified that Deacetylase inhibitors treated cells displayed a rapidly accumulating level of Drosha protein (Tang et al., 2013). In line with similar mechanism, ERK/MAPK mediated phosphorylation of DGCR8 increased its stability (Herbert et al., 2013). The product formed as a result of processing by Drosha-DGCR8 is a precursor hairpin structure of 60-70 nucleotides, pre-miRNA. In cytoplasm, pre-miRNA is transformed to mature single-stranded miRNA (ss miRNA) by Dicer (RNase III) and is finally incorporated into the RNA-induced silencing complex (RISC) where it guides RISC to silence target mRNAs through mRNA cleavage, translational repression or deadenylation, whereas the passenger strand is degraded. Post-transcriptional regulation of Dicer is also an essential mechanism. Hippo pathway effectors TAZ and YAP (TAZ/YAP) regulate post-transcriptional modulation of Dicer via suppression of Let-7 (Chaulk et al., 2013). Transcriptional regulation of Dicer by GATA has also been recently studied in leukemic cells and it was found that gene silencing of Dicer induced apoptosis in leukemic cells (Bai et al., 2013). Dicer is often unable to process 5' methylated pre-miRNA molecules. There is a recent report that underscores inability of Dicer to process 5' methylated pre-miR-145 (Xhemalce et al., 2012). In the upcoming sections we attempt to provide an overview of the recent advancements in understanding of roles of miR-421, mir-155 and miR-650.

miR-421 in Cancer

Increasingly it is being recognized that miR-421 is a potential diagnostic marker for gastric carcinoma. In-vitro studies have shown that targeted inhibition of miR-421 using antagomir resulted in considerable regression of

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growth of both MGC-803 and SGC-7901 gastric cancer cells. In addition, target genes of miR-421 including CBX7 and RBMXL1 were upregulated in miR-421 silenced cancer cells (Jiang et al., 2010). Targeted inhibition of miR-421 has also been studied animal models and it has been shown that there was a tumor regression. It is worth mentioning that miR-421 has recently been indicated as biomarker for monitoring circulating tumor cells in gastric cancer patients (Zhou et al., 2012). miR-421 has recently been shown to induce resistance to apoptosis in nasopharyngeal carcinoma cells via targeting of FOXO4 (Chen et al., 2013).

miRNA Regulation of ATM

It has previously been convincingly revealed that miR-421 is involved in quantitative control of miR-421. In-vitro assays verified the fact that miR-421 is triggered by a transcription factor, N-Myc and negatively regulates ATM. Hela cells were reconstituted with miR-421 and protein assays revealed remarkably reduced protein expression of ATM. Interestingly, kinase activity of ATM was determined using a downstream substrate pS966-SMC1. It was noted that miR-421 transfected Hela cells displayed notably reduced pSMC. Moreover, Hela cells over-expressing miR-421 were radio-sensitive (Hu et al., 2010). Similar mechanism was studied in squamous cell carcinoma and it has lately been indicated that miR-421 over-expressing cells were significantly more sensitive to radiation. Interference with miR-421 using antagomirs or microRNA-insensitive expression vector for ATM dramatically inhibited hyper-sensitivity to radiations (Mansour et al., 2013).

miR-421 Controls FXR and SMAD4

There is emerging evidence that suggests importance of farnesoid X receptor (FXR) in suppressing cell proliferation and migration. However FXR is negatively regulated by miR-421 and biliary tract cancer cells over-expressing miR-421 revealed a dramatic increase in proliferation and migration of the cells (Zhong et al., 2012). MiR-421 mediated control of FXR has also been studied in hepatocellular carcinoma cells and similar characteristics were noted upon transient transfection of miR-421 in hepatocellular carcinoma cells (Zhang et al., 2012). SMAD4 is negatively regulated by miR-421 in pancreatic cancer cells (Hao et al., 2011).

miR-155 Regulation of Wnt Signaling

Increasingly it is being recognized that miR-155 negatively regulates Adenomatous Polyposis Coli (APC). Targeted inhibition of APC by miR-155 rescued β-catenin. Additionally there was a marked increase in various oncogenes including c-Myc, cyclin D1, TCF-1 and LEF-1 (Zhang et al., 2013). In hepatocellular carcinoma it had been shown that miR-155 triggered by NFKB consequently promoted Wnt signaling as evidenced by increased transportation of catenin into the nucleus (Zhang et al., 2012).

Targets of miR-155

It has been persuasively revealed in ovarian cancer initiating cells (OCIC) that miR-155 negatively regulates claudin-1. Surprisingly, growth of OCIC xenograft tumors was also notably reduced via miR-155 over-expressing cancer cells (Qin et al., 2013). However the existing mechanism of Claudin-1 negative regulation by miR-155 is challenged by a recent report that revealed upregulation of claudin-1 in miR-155 over-expressing colorectal cancer cells (Zhang et al., 2013). miR-155 has been noted to down regulate Sel-1-like (SEL1L) in pancreatic ductal adenocarcinoma (Liu et al., 2013). FOXO3 has also been shown to be controlled by miR-155 in glioma cells (Ling et al., 2013). Mut L homologue 1 (MLH1) expression is directly related to level of differentiation in pancreatic cancer cells. However MLH1 is a direct target of miR-155 and it is worth mentioning that miR-155 mediated MLH1 down-regulation resulted in lack of differentiation (Liu et al., 2013).

Regulation of miR-155

miR-155 was considerably higher in poorly differentiated cancer cells as compared to more differentiated cancer cells (Zhao et al., 2013). Mounting evidence suggests that JAK-STAT signaling is also potentiated in miR-155 over-expressing cancer cells via negative regulation of SOCS1. Experimental data revealed that level of expression was directly linked to degree of differentiation of cancer cells. It is noteworthy that STAT3 binding sites are present in promoter region of miR-155 (Rozovski et al., 2013). Subsequent study provided proof of the concept that STAT3 silenced cells displayed considerably reduced expression of miR-155 construct that contained 700-709 bp STAT3 binding site (Li et al., 2013). It is appealing to note that STAT5 is also involved in stimulating the expression of miR-155 in cutaneous T-cell lymphoma (Kopp et al., 2013). It is getting increasingly clear that miR-155 is involved in downregulation of CCAAT-enhancer binding protein beta (C/EBPβ). C/EBPβ mediated expression of E-cadherin and therefore loss of C/EBP resulted in epithelial to mesenchymal transition (Johansson et al., 2013). There is a direct piece of evidence that suggests that miR-155 is controlled by SMAD4 in TGF treated breast cancer cells (Kong et al., 2008). mutant p53-expressing tumors displayed substantially enhanced expression of miR-155 (Neilsen et al., 2013).

miR-155: *in vitro* Studies

It has recently been convincingly revealed that silvestrol considerably repressed FLT3 protein expression and miR-155 in FLT3-wt overexpressing (THP-1) and FLT3-ITD (MV4-11) expressing cancer cells (Alachkar et al., 2013). It has been experimentally verified that ectopically expressing miR-155 in mice B cells (Eμ-miR-155 transgenic mice) induced pre-B-cell proliferation. Detailed mechanistic insights revealed that miR-155 directly targeted HDAC4, a corepressor

partner of BCL6. Ecotopically expressing HDAC4 in DLBCL cells considerably impaired miR-155-induced proliferation (Sandhu et al., 2012).

It has also been shown that camptothecin treated cancer cells represented a marked decrease in protein expression of HIF-1α. Mechanistically it was reported that camptothecin exerted its inhibitory effects via upregulating expression of miR-155 (Bertozzi et al., 2014).

Adrenaline has been shown to trigger expression of miR-155 in HT29 colon cancer cells, via NFKB. miR-155 overexpressing HT29 cells displayed a cisplatin resistant phenotype (Pu et al., 2012). Estradiol (E2) stimulated expression of miR-155 in MCF-7 cells. Additionally it was shown that transfecting miR-155 inhibitors in MCF-7 cells induced apoptosis (Zhang et al., 2013). Another contemporary study highlights cancer promoting role of miR-155 in MDA-MB-157 cells. Transfecting MDA-MB-157 cells with miR-155 antisense oligonucleotides (ASO) considerably increased apoptotic rate. More interestingly transplanting ASO expressing MDA-MB-157 cells in mice inhibited growth of tumor (Zheng et al., 2013). For enhancing the delivery and distribution of antisense against miR-155, polymeric nanoparticles encapsulated antisense peptide nucleic acids have been used (Babar et al., 2012).

It is surprising to note that tumor initiating cells expressing CD133 and CD338 show upregulated expression of miR-155 (Yao et al., 2014). Prognostic role of miR-155 in non-small cell lung cancer patients has also been reported (Xu et al., 2013).

miR-650

miR-650 has emerged as an oncomir as evidenced by in-vitro experiments. It has been shown that immunoglobulin gene rearrangement upregulates the expression of miR-650 in chronic lymphocytic leukemia (Mraz et al., 2012). Inhibitor of Growth 4 (ING4) is a tumor suppressor and a target of miR-650 both in gastric cancer and hepatocellular carcinoma (Zhang et al., 2010; Zeng et al., 2013) . N-myc downstream-regulated gene 2 (NDRG2) is also a direct target of miR-650 in colorectal cancer cells (Feng et al., 2011).

Long Non Coding RNAs

It is getting increasingly clear that long non-coding RNAs have regulatory role in carcinogenesis. It has lately been shown that maternally expressed gene 3 (MEG3) encodes a lncRNA. MEG is a tumor suppressor and experimental data suggests that MEG3 silenced cells demonstrated a greater cell proliferation. Contrarily, cells reconstituted with MEG3 indicated a marked decrease in cellular proliferation (Sun et al., 2013). In line with this concept, evidence has started to shed light on the fact that there are some other lncRNAs which are involved in enhancing proliferation potential of cancer cells. Targeted inhibition of lncRNA, HNF1A-AS1 in oesophageal adenocarcinoma cells resulted in suppression of cell proliferation (Yang et al., 2013). Substantial fraction of information has been added into the existing pool of targets of miRNAs. Accordingly, a recent finding provides persuasive evidence of miR-21 regulation of a lncRNA growth arrest-specific 5 (GAS5). It is of particular note that GAS5 also controls the expression of miR-21 (Zhang et al., 2013). HOX transcript antisense RNA (HOTAIR) gene encodes lncRNA. The gene is localized in Homeobox C (HOXC) gene cluster. HOTAIR was negatively regulated by miR-34 in prostate cancer cells. Genistein treated prostate cancer cells revealed down-regulated HOTAIR (Chiyomaru et al., 2013). Androgen receptor mediated transcriptional regulation of target genes is enhanced by lncRNAs in prostate cancer cells. In-vitro assays indicated that PRNCR1 (PCAT8) and PCGEM1 were found to be associated with androgen receptor (Yang et al., 2013).

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