miR-221: a critical player in apoptosis as a target of Caspase-3

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Caspases are a family of proteases that function as the primary mediators of apoptosis. miR-221 is an oncogenic miRNA that may have both anti-angiogenic and angiogenic effect. The aim of this work was to compare the expression levels of miR-221 and its target caspase-3 in different cancer cell lines and to find out a relationship between these two. We also analyzed binding efficiency of miR-221 to 3'UTR region of CASP3 gene computationally. Our results indicate that expression of caspase-3 is quite lower as compared to miR-221 expression in all of the selected cancer cell lines. Also, our computational results showed that miR-221-3p, of which binding site has two SNPs (rs369703524, C/T; rs189187745, T/A) on CASP3 3’UTR region. As a result, we conclude that miR-221 may have a crucial role in repressing the expression of caspase-3 which may conduce to a lower level of apoptosis, therefore supporting to select more aggressive cancer cells.

Keywords: miR-221; caspase-3; apoptosis; cancer cell line


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Introduction

Apoptosis, a programmed cell death phenomenon, occurs in human cells via two important pathways which are termed as the intrinsic and extrinsic cascades. The intrinsic (i.e. mitochondria-mediated) pathway is responsible for cytochrome-c releasing from mitochondria leading to the apoptosome structure construction, consisting of procaspase-9, cytochrome-c and Apaf-1 that gives rise to caspase-9 activation, then triggering caspase-3 and thus DNA fragmentation finally [1, 2]. During this pathway, outer mitochondrial membrane loses its integrity in a process termed outer Mitochondrial Membrane Permeabilization (MOMP) that causes OMI (HTRA2) and Second Mitochondria-Derived Activator of Caspase (SMAC or DIABLO) release. These proteins increase the caspase activity by preventing caspase inhibition by inhibitor of apoptosis proteins (IAPs). IAPs, including XIAP and survivin, take a crucial function in suppression of apoptosis in cancer cells [3].

Apoptosis is a strictly organized course having a crucial function in homeostasis and development. The organization of apoptosis is designed genetically and apoptosis has an important act in directing disease, development and growth. Apoptosis involves DNA disintegration, blebbing, and crumbling of the cell [4, 5]. At the time aberrant apoptosis or disorganization of apoptosis
occurs, many hazardous clinical outcome may result, like cancer and neurodegenerative or autoimmune illnesses [6,7,8].

Apoptosis is triggered by apoptosis-related signaling via caspase-linked cascades. Cytokines/Fas-triggered cascades, mitochondrial-triggered cascades, and endoplasmic reticulum (ER)/Ca2+-triggered cascades are among them [9,10,11]. Fas or cytokines (e.g., TNF-α, IL-1β) binding to their cell membrane receptors launch apoptotic cascade via collection of adaptor proteins (e.g., Fas-associated death domain; FADD). The constructed death-stimulating signal structure provides activation of caspase-8, which then triggers caspase-3 activation [9].

Caspases are a series of protein-cutting enzymes which are cysteine dependent aspartate-specific proteases [12,13]. So far, 14 caspases directing to cell death via apoptosis have been found in mammals [14]. Caspases takes crucial functions in apoptosis-related cascades and interact the non-caspase apoptotic cascade with the inclusion of apoptosis inducing factor (AIF) and endonuclease G (Endo G) [15]. Cytochrome c interacts with Apaf-1 (apoptotic protease-activating factor 1) and pro-caspase-9 to build up the apoptosome to activate both caspase-3 and caspase-9, ensuing apoptosis via DNA disintegration [16].

Apoptotic cell can use both extrinsic (death ligand) and intrinsic (mitochondrial) cascades in order to activate caspase-3. Caspase-3 has to possess the zymogen characteristics since if it is deregulated, the activity of the caspase kill cells caspase activity would kill cells promiscuously. After apoptotic signaling incidents have realized and the caspase-3 zymogen is cleaved by a starter caspase, the executioner caspase-3 zymogen finally shows its activity [17,18,44].

microRNAs (miRNAs) are a group of 22~25 nucleotide non-coding RNAs that takes a crucial function in organization of different biological functions. miRNAs bind to complementary sequences in the 3′-untranslated region (3′-UTR) of the target gene transcripts and regulate their gene expression and function including apoptosis of tumor cells. miRNA can function as tumor suppressors and oncogenes. Dysregulation of miRNA expression has been reported in different human cancers including colon cancer, breast cancer, prostate cancer, hepatocellular carcinoma, and osteosarcoma. Intensive studies during the last several years have identified numerous affected miRNAs in association with apoptosis, their target genes and biological functions, and possible drug interventions. For example, apoptosis is triggered by miR-15a and miR-16-1 (miR-15a/16-1) over mitochondrial function’s regulation. They are found as a cluster at the chromosomal region 13q14 and are often down-regulated or deleted in chronic lymphocytic leukemia (CLL). MiR-1, a muscle specific miRNA, expression is raised with an accompanying cytochrome c release through mitochondria and a descend in membrane potential upon apoptotic activation [19,20].

MiRNAs bind to target sequences (usually located in the 3′ untranslated region [3′UTR]) in messenger RNAs (mRNAs) and act by negatively regulating gene expression. This binding requires complementarity between the nucleotides 2-8 of miRNA (named as “seed” area) and the target mRNA. To date more than 2500 mature human miRNAs have been specified and they are predicted to regulate over 60% of human protein-coding genes. This regulatory network can be very complex as one miRNA may potentially regulate several mRNAs, and a given mRNA may possess in its sequence binding sites for several miRNAs [21,22].

Specific miRNA expression signatures can accurately discriminate different diseases, especially cancer, and are often of great prognostic relevance. Changes in miRNA expression may result from genomic and epigenetic alterations or the impairment of miRNA biogenesis pathway. In addition, polymorphisms in miRNA genes or miRNA target sites (miRSNPs) can modify miRNA action. While polymorphisms in miRNA genes are relatively rare, SNPs in miRNA-binding sites in target genes are more frequent. Several studies have shown that SNPs in miRNA target sites enhance or weaken the interaction between miRNA and its target transcripts and are associated with cancers and other diseases [23,24,25].

8 different cell lines were studied for their caspase-3 and miR-221 expressions. We calculated Ct values of caspase-3 and miR-221 of these cell lines as relative expression in Real-Time PCR. According to relative expressions of caspase-3 and miR-221 in cell lines calculated with 2-Δct method, it was observed that miR-221 expression was higher as compared to caspase-3 expression in all selected cell lines such as SKBR3 (breast cancer), HCC1500 (breast cancer), MDA-MB-231 (breast cancer), A549 (lung cancer), DU145 (prostate cancer), HepG2 (hepatocellular carcinoma), HeLa (cervix cancer) and HGC-27 (gastric cancer).

MiR-221 has been dedicated as promoter of a thrusting basal-like characteristic of breast cancer according to a study freshly performed. These show the roles of miR-221 in breast cancer progression [26-28]. The result obtained in our study also shows an increased expression of miR-221 and a decreased caspase-3 expression in several cell lines of breast cancer like SKBR3, CRL1500, MDA-MB-231. The differences between miR-221 and caspase-3 in these three cell lines were statistically significant (p<0.05). The decreased expression of caspase-3 as compared to miR-221 indicates the role of this miRNA in breast cancer cell progression via inhibiting apoptosis. According to another study, it has been reported that oncogeneity and tumorigenesis regulation was among the roles of miR-221.
in triple-negative breast cancers (TNBCs) in vivo and vitro studies. Down regulation of miR-221 inhibited the improvement of cell cycle, triggered apoptosis of the cell and blocked the proliferation of the cell in-vitro however no suggestion was done for the mechanism of those upon the findings [29]. Our study completes this study finding with results obtained from TNBC cell line, MDA-MB-231, because we can supplement our and that study’s results like that miR-221 inhibits apoptosis by targeting and blocking caspase-3 expression.

When we want to compare the relationship between caspase-3 and miR-221 in breast cancer cell lines in our study with respect to their different histopathological characteristics, we see they have different hormone receptor status, especially for ER, PR and HER2. The difference between the expressions of miR-122 and caspase-3 was lower in our only HER2+ breast cancer cell line, SKBR3, compared to our HER2- breast cancer cell lines. Also, the expression levels of them were also lower in SKBR3 than other two. Moreover, in TNBC cell line, MDA-MB-231, oncogenic miR-221 expression level and the difference between miR-221 and caspase-3 expressions were higher than other two breast cancer cell lines. Its tumor grade is higher than other two so high miR-221 expression is related to this fact.

The expression of miR-221 in contrast to caspase-3 is also elevated in lung cancer cell line, A549. The difference between miR-221 and caspase-3 in this cell line was statistically significant (p<0.05). Our result also holds true for lung cancer as another study has clear evidence that miR-221 has a prominent role in lung cancer progression [30,31]. According to another study, downregulation of miR-221 somehow in lung cancer increases the protein level of caspase-3, resulting in an increase in gefitinib-induced apoptosis [32]. This finding totally supports our finding related to miR-221 and caspase-3 roles for apoptosis in lung cancer.

The prostate cancer cell line (DU145) also showed an increased over expression of miR-221 than caspase-3 suggesting a role of miR-221 in prostate cancer development via inhibiting caspase-3 activity. The role of miR-221 in prostate cancer is also discussed in many research works which totally validates our finding [33-36]. Increased level of miR-221 expression alters the expression of a lot of genes found in cell cycle and cascades triggering the transition from epithelial to mesenchymal transition and metastasis of tumor is induced [34]. Also, the potency of human prostate cancer cell lines to invade is influenced by the expression of miR-221 targeting DVL2 [35]. Our study results shows in the same line with these studies that prostate cancer progression activated by miR-221 is caused by targeting of caspase-3 and inhibiting apoptosis. Some study contradicts the increased expression of miR-221 in prostate cancer cell line [37]. However, in that study, miR-221 downregulation was seen in a very specific and limited prostate cancer population having tumors bearing TMPRSS2: ERG fusion transcripts. So, that study cannot give a general view about miR-221 expression profile in prostate cancer.

The statistically significant increase in expression of miR-221 in hepatocellular carcinoma cell line, HepG2, as compared to caspase-3 indicates that miR-221 has carcinogenic role by regulating caspase-3 expression level (p<0.05). This particular finding complies with the experiment carried out by Laura Gramantieri et al. which states that miR-221 inhibits apoptosis by targeting Bmf [38]. Also, according to the same study, they proved that a more aggressive characteristic of hepatocellular carcinoma is related to the over-expression of miR-221. So, upon this finding, we can also say that increased miR-221 in our results is the sign of aggressiveness of HepG2.

There was also a slight increase of miR-221 expression in cervical cancer cell line (HeLa) with respect to caspase-3 which further matches to the findings in other literature showing over expression of miR-221 in cervical cancer [39]. Our study supports this study by proving miR-221 over expression with caspase-3 down regulation and shedding light the mechanism how miR-221 cause cervical cancer progression.

An upregulation of miR-221 in gastric cancer also indicates similar result explained recently by Wang M et al. According to that study, miR-221 delivery to HGC-27 cell line via exosomes promotes its proliferation and migration and also the inhibition via miR-221 targeting in HGC-27 may inhibit its tumor progressive characteristic [40]. As an addition from our study to that research, this blocking of tumor related behavior occurs through activation of caspase-3 which is target of miR-221 and so apoptosis.

In other studies upon different cell lines than in our study, in human glioma cells, cell apoptosis is blocked by targeted inhibition of pro-apoptotic PUMA gene by miR-221 [42]. MiR-221 is also found to have tumor-promoting activity following prevention of the expression of the tumor suppressor p27kip1 in glioblastoma [41]. MiR-221 is also highly expressed in atypical teratoid/rhabdoid tumors [43]. All in all, our study clearly highlights oncogenic role of miR-221 in almost majority of the selected cancer cell lines of different cancer groups. This is most probably done by down regulating caspase-3 expression having apoptotic activity.

Moreover, we analyzed the targeting of miR-221 to 3’UTR region of CASP3 gene according to five different databases: DIANA Tools, miRTarBase, miRanda, Targets can, STarMir. MiR-221-3p, of which binding site has two SNPs (rs369703524, C/T; rs189187745, T/A) on CASP3
3'UTR, has a crucial role in repressing the expression of caspase-3 which may conduce to a lower apoptotic ratio, therefore to select more aggressive cancer cells. Moreover, the down regulation of miR-221-3p to increase the protein level of caspase-3 results in an increase in gefitinib-triggered apoptosis in non-small-cell lung cancer (NSCLC) cell lines. Further, the analysis of HCC tissues showed an opposite association between miR-221-3p and activated caspase-3, as a sign of apoptosis. Else, down regulated miR-221-3p, targeting caspase-3, can sensitize glioma cells to Temozolomide (TMZ) by adjusting apoptosis free from p53 status. Besides, miR-221-3p, by targeting Bmf, obstructs apoptosis in HCC and miR-221-3p over expression is correlated with a more offensive phenotype [2].

Moreover, miR-221 also targets apoptosis related genes other than CASP3 according to Mir Tar Base database. These genes are TNFSF10 and TP53. This means that miR-221 shows its effect on apoptosis through these two genes, besides CASP3.

Conclusions

Breast cancer cell lines (SKBR3, HCC1500, MDA-MB-231), lung cancer cell line (A549), prostate cancer cell line (DU145), hepatocellular carcinoma (HepG2), cervix cancer cell line (HeLa) and gastric cancer cell line (HGC-27) showed increased expression of miR-221 with decreased expression of caspase-3. It is asserted that overexpressing miR-221 in cancer cell lines increases tumor progression via downregulation of the expression of caspase-3. This may be the explanation of their functions in tumor formation in many cancers. Our discoveries indicate that many human cancer cell lines undergo changes in miR-221 and caspase-3 expression that can help to explain the oncogenic property of miR-221 via inhibiting apoptotic activity of caspase-3. We may say that, by inhibiting caspase-3 expression, high miR-221 levels might conduce lowering the ratio of apoptosis, therefore favoring the selection of more aggressive cancer cells, which can so cause multifocal tumors. All in all, our findings is directed toward further defining which other factors regulates caspase-3, so apoptosis. We will use this crucial information to develop novel therapies to treat diseases in which caspase-3 plays an important role, including human cancer metastasis.

Conflict of interest

Authors have no conflict of interest regarding the subject of this manuscript.

References


