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Research article

Sequence-based analysis of 5′UTR and coding regions of *CASP3* in terms of miRSNPs and SNPs in targetting miRNAs

Sercan Ergun[a,*], Serdar Oztuzcu[b]

[a] Ulubey Vocational Higher School, Ordu University, Ordu, Turkey
[b] Department of Medical Biology, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey

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ABSTRACT

Apoptosis is described as a mechanism of cell death occurring after adequate cellular harm. Deregulation of apoptosis occurs in many human conditions such as autoimmune disorders, ischemic damage, neurodegenerative diseases and different cancer types. Information relating miRNAs to cancer is increasing. miRNAs can affect development of cancer via many different pathways, including apoptosis. Polymorphisms in miRNA genes or miRNA target sites (miRSNPs) can change miRNA activity. Although polymorphisms in miRNA genes are very uncommon, SNPs in miRNA-binding sites of target genes are quite common. Many researches have revealed that SNPs in miRNA target sites improve or decrease the efficacy of the interaction between miRNAs and their target genes. Our aim was to specify miRSNPs on *CASP3* gene (caspase-3) and SNPs in miRNA genes targeting 5′UTR and coding exons of *CASP3*, and evaluate the effect of these miRSNPs and SNPs of miRNA genes with respect to apoptosis. We detected 141 different miRNA binding sites (126 different miRNAs) and 7 different SNPs in binding sites of miRNA in 5′UTR and CDS of *CASP3* gene. Intriguingly, mi*R*-339-3p's binding site on *CASP3* has a SNP (rs35372903, G/A) on *CASP3* 5′UTR and its genomic sequence has a SNP (rs565188493, G/A) at the same nucleotide with rs35372903. Also, miR-339-3p has two other SNPs (rs373011663, C/T rs72631820, A/G) of which the first is positioned at the binding site. Here, miRSNP (rs35372903) at *CASP3* 5′UTR and SNP (rs565188493) at miR-339-3p genomic sequence cross-matches at the same site of binding region. Besides, miR-339-3p targets many apoptosis related genes (*ZNF346, TAOK2, PIM2, HIP1, BBC3, TNFRSF25*, CLCF1, IHPK2, NOL3) although it had no apoptosis related interaction proven before. This means that miR-339-3p may also have a critical effect on apoptosis via different pathways other than caspase-3. Hence, we can deduce that this is the first study demonstrating a powerful association between miR-339-3p and apoptosis upon computational analysis.

1. Introduction

A process including cell suicide known as apoptosis gets rid of distorted cells in the body. Inhibition of apoptosis is very crucial for tumor initiation and progression because apoptosis ordinarily eliminates cells with high malignant potential, such as those with distorted DNA or abnormal cell cycling. Fundamental cancer research has provided striking developments in our comprehension on cancer biology and genetics in the last decade. The most crucial advance is to realize that apoptosis and the genes controlling it have a deep impact on the malignant phenotype. For instance, it is currently evident that some oncogenic mutations destroy apoptosis, causing tumorigenesis. However, extensive evidence is also accumulating that other oncogenic alterations may trigger apoptosis, underlining the fact that apoptosis is suppressed by selective forces acting on a multi-pathway tumorigenesis process (Lowel and Lin, 2000).

Two main cell-based mechanisms have been defined for apoptosis initiation, one starting at the level of death receptors of cell surface and another one including mitochondria activation tracked by cytochrome c releasing. The initiation of these cascades induces the activation of caspase pathways. Up to now, 14 distinct caspases have been defined. Despite the fact that upregulation of any one of them can cause cell death via apoptosis, none of them is not ordinarily included in this operation. The main starter caspases are caspases-8 and caspase-9. Binding of death ligands to their related receptors induces caspase-8 activation, however caspase-9 is triggered by releasing of mitochondrial cytochrome c and the

* Corresponding author.
E-mail address: sercanergun@msn.com (S. Ergun).

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following apoptosome complex formation. The main effector caspases are caspases-3, −6 and −7 and they cut many proteins undergoing proteolysis in apoptotic cells when they are activated (Thyrell et al., 2002).

Caspases are considered as important intermediary elements in programmed cell death (apoptosis). As final mediator, caspase-3 is a mostly activated death protease, facilitating the particular cleavage of many crucial cellular proteins. But, the particular necessities of this caspase have stayed mainly mysterious in apoptosis by now. Cascades involved in caspase-3 activation have been described as dependent on or independent of mitochondrial cytochrome c release and caspase-9 function. Caspase-3 is necessary for regular brain development and is crucial or requisite in other apoptotic mechanisms in a considerable tissue-, cell type- or death signal-specific way. Caspase-3 is further needed for some ordinary characteristics of apoptosis, and is inevitable for DNA breakup and apoptotic chromatin condensation in all cell types studied. Therefore, caspase-3 is necessary for some functions related with the disassembly of the cell and the apoptotic body formation however it may further work before or at the step when dedication to loss of cell viability is done (Porter and Jänicke, 1999).

Caspase-3 is executioner caspase and has zymogen characteristics, that is, has to be activated. Apoptotic cell can use both extrinsic (death ligand) and intrinsic (mitochondrial) cascades in order to activate caspase-3. Until caspase-3 activation (during other caspases' functions), apoptosis can be reversed or suppressed. However, after it is activated, it works in an irreversible manner. Other caspases can be regulated back but caspase-3 cannot be. Caspase-3 is indispensable for apoptotic chromatin condensation and DNA fragmentation in all cell types. Also, other caspases taking role in apoptosis can be regulated by many different ways but caspase-3 is most prominently regulated by post-transcriptional manner (especially RNAi). (Ergun and Oztuzcu, 2014). For these reasons, caspase-3 was selected to study apoptosis.

MicroRNAs (miRNAs) are small non-coding RNAs regulating gene expression negatively which are about 18–24 nucleotides in length. miRNA can work as oncogenes and tumor suppressors. Dysregulation of miRNA expression has been declared in various human cancers containing prostate cancer, breast cancer, colon 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, let-7a is related with apoptosis via targeting caspase-3 directly. Moreover, miR-34 family members targets p53 directly, and their overexpression triggers cell cycle arrest and apoptosis. Hereof, miR-34a has been depicted to regulate genes taking role in cell cycle control and apoptosis, containing *E2F3*, cyclin-dependent kinase 4 (*CDK4*), *CCND1*, *SIRT* and *CDK6*. Futhermore, miR-16 is concerned with triggering of apoptosis via targeting Bcl-2, and is included in regulation of the cell cycle via targeting cell division cycle protein 27 (*CDC27*), *CDK6*, cyclin E, the caspase recruitment domain-containing protein 10 (*CARD10*) and *CCND1* (Aranha et al., 2010).

It has been recently shown that mammalian miRNA targeting doesnot always limit itself to 3′ miRNA action. It now seems that in addition to the traditional 3′ UTR targeting, mammals are starting to look a bit more like plants as they also target a number of the 5′ untranslated region (5′ UTR) and amino acid coding sequence (CDS) sites (Zhou and Rigoutsos, 2014). An elevated percentage (25%) of the reads were mapped to open reading frames (ORFs), even though the most matched to 3′ UTRs and just 1% to the 5′ UTR, affirming that miRNAs rather bind their targets via the 3′ UTR but also emphasizing the significance of CDS-mediated interactions. Likewise, one of the largest restrictions of available databases is that the estimations are mostly limited to the 3′ UTRs, when the latest experimental throughputs show that most of the miRNA/mRNA interactions may happen via CDSs or even the 5′ UTR. Moreover, the binding rules for miRNA/target interactions via the 3′ UTR may differ via other mRNA regions, also decreasing the capacity of available databases to estimate these interactions (Martinez-Sanchez and Murphy, 2013).

The plethora of published reports in recent years demonstrate that miRNAs play fundamental roles in many biological processes, such as carcinogenesis, angiogenesis, programmed cell death, cell proliferation, invasion, migration, and differentiation by acting as tumor suppressor or oncogene, and aberrations in their expressions have been linked to onset and progression of various cancers. Furthermore, each miRNA is capable of regulating the expression of many genes, allowing them to simultaneously regulate multiple cellular signalling pathways. Hence, miRNAs have the potential to be used as biomarkers for cancer diagnosis and prognosis as well as therapeutic targets. Also, dysregulation of miRNA networks has been implicated in biological processes specified above. One of the reasons for disturbed miRNA-mediated regulation is polymorphism in miRNA-binding sites (miRSNPs), which alter the strength of miRNA interaction with target transcripts (Dzikiewicz-Krawczyk, 2014). Moreover, common genetic variants like single nucleotide polymorphisms (SNPs) in miRNA genes could change their expression or maturation ensuing changed functional outcomes in carcinogenesis (Bansal et al., 2014). Many studies have revelaed that SNPs in miRNA target sites strengthen or weaken the interaction between miRNA and its target transcripts and are related to cancers and other diseases (Melo and Esteller, 2011; Salzman and Weidhaas, 2013).

Considering that miRNAs have been shown to play an essential role in apoptosis and that SNPs in miRNA-binding sites in target genes have been related to many cancers, in this study, we took aim at defining miRSNPs on executioner caspase, *CASP3* gene (caspase-3) and SNPs in miRNAs genes targeting 5′UTR and coding regions of *CASP3* and evaluating the effect of these miRSNPs and SNPs of miRNA genes targeting 5′UTR and coding regions of *CASP3*in terms of apoptosis.

2. Materials and methods

2.1. Screening of miRNA targeting 5′UTR and CDS of CASP3 gene

Two different online databases, miRWalk and miRcode, were used in order to investigate which miRNAs targets 5′UTR and coding regions of *CASP3* gene. The common characteristic of these two databases is the fact that they take into account 5′UTR and coding regions of targeted genes. Also, miRó database was used to associate specific miRNAs with apoptosis-related genes. Three different databases were used instead of only one database because all these databases uses different algorithms and computational approaches so we considered that we could achieve to screen all miRNAs targets without skipping any of them by using different databases.

2.1.1. miRWalk

Information about miRNA-target interactions is generated through miRWalk algorithm on the total sequence (promoter, 5′ UTR, CDS and 3′ UTR) of all known genes of Human, Mouse and Rat containing all transcripts. Predicted miRNA-target interactions gives information on genes related with 449 human biological cascades and 2356 OMIM diseases. The information is provided on 3 total mitochonrial genomes i.e. Human, Mouse and Rat. MiRNA interactions related with genes, organs, pathways, transcription factors and diseases are experimentally verified or predicted. It further gives approved miRNA interactions information on cell