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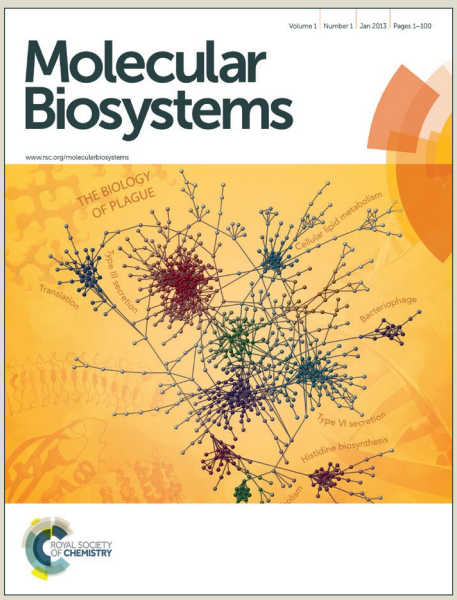

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The Regulatory Function of MicroRNA-1 in Arrhythmias

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Arrhythmia, the basis of which is cardiomyocyte ion channel abnormalities, poses a serious threat to human health. A large number of studies have demonstrated that miRNA-1(miR-1) is involved in the occurrence of arrhythmia in many myocardial pathological conditions by post-transcriptionally regulating a variety of ion channels and proteins related to cardiac electrical activity. We aim at emphasizing the relationship between miR-1 and ion channels and proteins involved in the process of arrhythmia. In addition, we will pay attention to its future therapeutic prospects.

Introduction

Arrhythmia is a frequent manifestation of cardiovascular diseases and poses a serious threat to human health. The electrophysiologic mechanisms responsible for arrhythmia are generally divided into classes of disorders of impulse formation, disorders of impulse conduction, or combinations of both. Disorders of impulse formation contain abnormal automaticity and triggered activity. Triggered activity is pacemaker activity that initiated by afterdepolarizations, which are depolarizing oscillations in membrane induced by preceding action potentials. These depolarizations can occur before or after repolarization and are termed early afterdepolarizations (EADs),when they arise from a reduced level of

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membrane potential during phases 2 and 3 of the cardic action potential, or delayed afterdepolarizations (DADs), when they occur after completion of repolarization (phase 4). When afterdepolarizations reach threshold potential, they can trigger the neighboring cells to induce an action potential or a series of action potentials, resulting in the occurrence of arrhythmia.[1, 2]

The coordinated activities of numerous cardiomyocyte ion channels, such as L-type calcium channels, transient outward potassium channel Kv4.2, hyperpolarization-activated cyclic nucleotide-gated cation channels (HCN), and inward rectifying potassium channel Kir2.1, underlie the maintenance of normal cardiomyocyte electrical activities. The dysregulation of these ion channels might lead to disruption of calcium current (ICa), transient outward potassium current (Ito), pacemaker current (If), and inward rectifier potassium current (IK1), and induce action potential duration (APD) alteration, leading to abnormal cardiomyocyte excitability, conductivity, and automaticity (Figure 1).[3]

MicroRNAs (miRNAs) are a kind of non-coding small RNA molecules consisting of ~22 nucleotides, which function as regulators by affecting the stability or repressing the translation process of the target gene mRNA.[4] Primary miRNAs (pri-miRs) are produced by RNA polymerase II (Pol II) and cropped into precursor miRNAs (pre-miRs) by Drosha. Finally, the pre-miRs are cleaved by the Rnase III enzyme Dicer to produce mature miRs. These mature miRs bind to mRNA targets and induce inhibition of target gene expression (Figure 2). Recently, numerous studies have found that miRNAs play a

pivotal role in physiology and development of muscle tissues including the heart. MiR-1 is specifically expressed in myocardium and is one of the most abundant miRNA in the heart, which is known to be involved in cardiogenesis, heart development, and cardiac pathological processes.[5-7] Many studies have reported that miR-1 has the responsibility in arrhythmogenesis via manipulating a variety of ion channels and proteins related to cardiac electrical activity in various myocardial pathological conditions.[8-12] The miR-1 family includes the miR-1 subfamily and miR-206. The miR-1 subfamily contains two transcripts, miR-1-1 and miR-1-2, which have the same mature products in humans, the common sequence is UGGAAUGUAAAGAAGUAUGUAU (www.microrna.org). However, miR-1-1 and miR-1-2 are located at chromosomal regions 20q13.33 and 18q11.2, respectively (Figure 3). The miR-1 genes are direct targets of essential transcription factors including serum response factor (SRF), MEF2 and MyoD. SRF sites in the miR-1-1 and miR-1-2 enhancers are almost identical and highly conserved in human, as were MEF2 sites in the miR-1-1 and MyoD sites in the miR-1-2. MiR-1-1 and miR-1-2 function in SRF-dependent pathways in cardiac progenitor cells, and are responsive to MEF2/MyoD in skeletal muscle precursors.

MiR-1 and cardiac conduction

The channels in which miR-1 is involved in regulating cardiac conduction mainly include the GJA1/Cx43, KCNJ2/Kir2.1/IK1, and Irx5/KCND2/Kv4.2/Ito.

Connexin 43 (Cx43) mainly forms the gap junction channels in ventricular cardiomyocytes and the low-resistance channels that accelerate electrical current

transmission between myocardial cells. Dysregulated Cx43 expression can cause a variety of ventricular arrhythmias. Cx43 knockout mice are susceptible to spontaneous ventricular arrhythmias, whereas Cx43 gene transfer improves conduction velocity and reduced ventricular tachycardia susceptibility in healed scar border zones following myocardial infarction (MI).[13, 14] Yang et al [15] reported higher miR-1 expression in patients with coronary artery disease(CAD) and in rat models with myocardial ischemia as compared to their respective controls and delivery of miR-1 into healthy hearts reduced Cx43 and Kir2.1 protein expression by respectively repressing the translation of GJA1 and KCNJ2, which were confirmed as the target genes of miR-1. The reduction of Cx43 slowed electrical conduction and Kir2.1 reduction attenuated IK1 and prolonged ventricular repolarization, leading to widened QRS complex and prolonged QT interval, resulting in increased incidence of premature ventricular beats and ventricular tachyarrhythmia. However, transfection anti-miR-1 oligonucleotides (AMO-1) abolished the downregulation of Cx43 and Kir2.1, reversed the above changes. Nevertheless, the effects of miR-1 in the above study are transient. Zhang et al[16] generated a miR-1 transgenic (Tg) mouse model with long-term miR-1 overexpression, and found that Cx43 and Kir2.1 protein levels were significantly reduced in ventricular myocytes from Tg mice. Additionally, they found frequent atrioventricular block (AVB) of varying degrees from miR-1 Tg mice, which might largely result from miR-1 overexpression. The channels involved in the AVB occurring were not made clear because of the technical difficulties.

Curcio et al[11] found that miR-1 expression was reduced in pressure overload-induced left ventricular hypertrophy (LVH) rat models, which increased Cx43 expression and phosphorylation correlated with its displacement from the gap junction. Consequently, ventricular effective refractory period (VERP) and monophasic action potential duration (MAPD) were significantly reduced, leading to the increased counts of premature ventricular contractions (PVC) (most of which were characterized by R-on-T phenomena) and induction of ventricular tachyarrhythmias. Furthermore, they found that valsartan reduced the occurrence of fatal ventricular arrhythmia, the mechanism for which partly relies on decreased Cx43 expression and reduced amount of phospho-Cx43 by upregulating miR-1 expression. Based on the abovementioned studies, it is easy to conclude that miR-1 regulates cardiac conduction by regulating Cx43 expression to a certain extent, that is, upregulating miR-1 would reduce Cx43 expression and slow electrical conduction, whereas, downregulating miR-1 would increase Cx43 expression levels and accelerate electrical conduction. Dysregulations of miR-1 and Cx43 are associated with increased susceptibility to ventricular arrhythmias.

Inward rectifying potassium channels (Kir) are a specific subset of potassium selective ion channels, which consist of seven subfamilies (Kir1.1~Kir7.1).[17] The Kir2.1 is encoded by the KCNJ2 gene. The reduced function of Kir2.1 may decrease the repolarization current, resulting in prolongation of action potential duration and QT interval. Numerous studies have demonstrated that miR-1 overexpression represses Kir2.1 expression and reduces IK1 density, contributing to the slowing of conduction

velocity, while downregulating miR-1 has the opposite effect. Yang et al[15] proved that overexpressing miR-1 reduced Kir2.1 expression by silencing its target ion channel gene KCNJ2 and reduced IK1 density, resulting in prolonged ventricular repolarization and consequently increased malignant ventricular arrhythmia. Recently, they also reported that miR-1 mediated the arsenic trioxide (As_2O_3)-induced cardiac electrical abnormalities. MiR-1 was significantly upregulated in guinea pig models established by intravenous injection with As_2O_3, leading to depression of Kir2.1 protein and IK1, which prolonged QT interval and QRS complex. Antisense inhibitors of miR-1 reversed all of the above changes.[18] Girmatsion et al[19] reported on reduced miR-1 expression in left atrial (LA) of patients with persistent atrial fibrillation (AF) , accompanied by upregulation of KCNJ2 mRNA and its protein Kir2.1, leading to increased IK1 which was important for AF maintenance.

Iroquois Homeobox domain 5 (Irx5) is a protein coding gene, which establishes the cardiac repolarization gradient by repressing the KCND2 (potassium voltage-gated channel subfamily D member 2). KCND2 encodes Kv4.2, the main pore-forming subunit for Ito (transient outward potassium current), which is the main current to the repolarizing phase 1 of the action potential.[20, 21] Zhao et al [22] discovered that mice that survived targeted deletion of miR-1 developed myocardial hypertrophy accompanied with arrhythmias. The mechanism was that the expression of Irx5, the target gene of miR-1, was greatly increased, while that of KCND2 was repressed, leading to weakened Ito current, as manifested by broad QRS complex waves on electrocardiogram. Myers et

al[23] discovered that Ito current was significantly reduced and APD was prolonged in mice ischemia-reperfusion (IR) mice model of MI, while miR-1 delivery prevented the increase in Irx5 expression and decrease in KCND2 levels, together with normalization of the Ito and APD.

MiR-1 and autorhythmicity

The pacemaker current If plays a critical role in automatic rhythmicity of cardiac pacemaker cells, and is regulated by hyperpolarization-activated cyclic nucleotide-gated (HCN) cation channels and mink- related proteins(K^+-channel-related protein, MiRP1). HCN channels are activated at negative membrane potentials upon repolarization and stimulated by intracellular cyclic nucleotides, leading to a depolarizing current which influences the threshold for subsequent AP generation.[24, 25] MiR-1 contributes significantly to myocardial autorhythmicity by regulating the HCN gene. Using a gene-specific microRNA mimic approach, Xiao et al[26] delivered miR-1 fragments into the ventricular myocytes of newborn rats. They found that the fragments effectively repressed HCN2 expression and reduced If channel conductivity and If density, contributing to the attenuation of autorhythmicity. Simultaneously, eliminating the inhibitory effect of endogenous miR-1 on HCN2 using a miRNA-masking antisense approach significantly increased HCN2 expression levels and functions, improving If channel conductivity and enhancing autorhythmicity. Luo et al[8] reported that, in LVH rats, miR-1 was decreased by 35% and HCN2 channel protein was increased by three times, leading to increased cardiac autorhythmicity and increased arrhythmia risk. Recently,

Alicia et al[27] found that the heart rates of rats and mice that had undergone standard exercise training were ~26% and ~20% lower, respectively, than that of the respective control animals. Their study showed that the heart rate adaptation to exercise training might have resulted from HCN4 downregulation and a consequent decrease in If density, which could have been driven by the significant upregulation of miR-1. Therefore, it is not difficult to conclude that downregulating miR-1 expression could be used to treat bradyarrhythmia, possibly by repairing the damaged sinoatrial node and conducting tissues; upregulating miR-1 expression might suppress ectopic excited nodes and it might be used to treat tachyarrhythmia.

MiR-1 and cardiac repolarization

The slow and rapid components of IK (IKs and IKr, respectively) are mainly involved in late action potential repolarization, which plays a vital role in maintaining APD. IKs, encoded by KCNE1 and KCNQ1,[28] helps maintain the repolarization reserve. Several studies have found that miR-1 alters the duration of repolarization mainly by influencing the level of KCNE1 expression. Li et al[29] found that miR-1 expression was augmented in human cardiac progenitor cells exposed to high levels of glucose and that KCNE1 expression was suppressed, following which IKs was significantly reduced. AMO-1 reversed the above changes, so authors believed that the dysregulation of miR-1 may partly underlie the mechanism of hyperglycemia-induced arrhythmias. Recently, Jia et al[30] reported that one-week right atrial tachypacing in New Zealand white rabbits increased miR-1 expression in an obvious manner, while that of KCNE1 was decreased.

As a result, the atrial IKs density was enhanced and the atrial effective refractory period (AERP) was shortened. Furthermore, KCNE1 expression was decreased more significantly when miR-1 was further upregulated through in vivo lentiviral infection, which induced higher atrial fibrillation inducibility with stronger IKs and shorter AERP. It was generally believed that the repression of KCNE1 should lead to increased IKs and prolonged APD as well as AERP. However, the researchers were unable to perform further investigations.

MiR-1 and cardiomyocyte calcium handling

Abnormal calcium handling can contribute to arrhythmogenesis directly by causing abnormal depolarizations and indirectly by regulating action potential time course and duration. The protein involved in the calcium handling during the excitation-contraction process in the heart are ryanodine receptor 2 (RyR2), sarcoplasmic reticulum Ca^{2+}-ATPase 2a (SERCA2a), sodium-calcium exchanger 1 (NCX1), L-type calcium channel, phospholamban, and calsequestrin. Among those proteins regulated by miR-1 responsible for arrhythmogenesis are RyR2 and NCX1.

Diastolic sarcoplasmic reticulum (SR) Ca^{2+}leaking gives rise to localized increases in the cytosolic calcium level that causing a propagating Ca^{2+} wave and depolarizes the cardiomyocyte membrane, triggering a DAD, which most likely play a causative role in arrhythmogenesis.[31, 32] RyR2 is a Ca^{2+} release channel in cardiac muscle SR and represents the main channel mediating the intracellular Ca^{2+} release. FKBP12.6, one major regulatory subunit interacting with RyR2, is the cytosolic FK506-binding protein

stabilizing the closed state of RyR2 channel and preventing diastolic Ca^{2+} leakage. The open probability of RyR2 is regulated by protein kinases, including protein kinase A (PKA), calcium/calmodulin-dependent protein kinase II (CaMK-II), and protein phosphatases PP1 and PP2A.[33, 34] Many studies have showed that increased RyR2 phosphorylation contributes to diastolic SR Ca^{2+} leak, resulting in DAD-triggered arrhythmias.[35, 36] Terentyev et al[37] indicated that miR-1 targets the regulatory subunit B56 of PP2A, which is decreased by miR-1 overexpression in ventricular myocytes, resulting in augmented phosphorylation of RyR2 by CaMK-II, leading to the increased sensitivity of RyR2 to calcium and increased incidence of arrhythmogenic spontaneous extrasystolic Ca^{2+} release and associated DADs. Belevych et al[9] reported significantly increased miR-1 levels in myocytes isolated from heart failure (HF) canine models and significantly decreased expression of B56α and B56δ, the regulatory subunits of PP2A, leading to PP2A dissociation from the RyR2 complex and CaMK-II-dependent hyperphosphorylation of RyR2, resulting in severe disruption of Ca^{2+}cycling and the occurrence of arrhythmia.

NCX1 is an expressed membrane protein essential in calcium homeostasis for many cells including those in mammalian heart and the main pathway for calcium efflux out of the cell in diastole. Kumarswamy et al [38] reported NCX1 as a new target protein of miR-1, they found that decreased miR-1 and the subsequent upregulation of NCX1 induced enhanced Ca^{2+} extrusion and faster Ca^{2+} transient in chronic post-MI HF models, while SERCA2a gene therapy increased miR-1 expression and decreased NCX1 expression via

a protein kinase B/forkhead box group O (Akt/FoxO3A)-dependent pathway, leading to the normalization of cellular Ca^{2+} concentrations. This SERCA2a-miR-1-dependent mechanism of NCX1 downregulation in HF might be responsible for the reduced incidence of DADs and arrhythmias, as it allowed more calcium to be re-sequestered into the SR.[39] Tritsch et al [40] also proved that NCX1 was a target of miR-1 through in vitro and in vivo manipulation of miR-1 expression levels and site-directed mutagenesis.

Prospects

Many new mechanisms of anti-arrhythmic drug therapies for arrhythmia have been reported. Lu et al [41] showed that propranolol downregulates miR-1 expression by inhibiting the β-adrenoceptor/cyclic adenosine monophosphate (-cAMP)/PKA signaling pathway and the expression of SRF, resulting in the upregulation of Kir2.1 and Cx43, which play an important role in alleviating myocardial ischemic injury and in treating arrhythmia. Tanshinone IIA reverses the downregulation of IK1 and its protein in myocardial ischemia by inhibiting SRF and reversing the overregulation of miR-1, which playes a profound role in treating arrhythmia and preventing sudden death.[42] These findings suggest that, as the indirect target of anti-arrhythmic drugs, miR-1 is the gateway for the development of new anti-arrhythmic drugs. An increasing number of studies have reported the successful regulation of target proteins expression by upregulating or downregulating miR-1 expression via miR-1 transfection or AMO-1 technology and eventually reversing disease states. Recently, with the discovery of natural carriers, such as exosomes and extracellular vesicles, the gene therapy has made a big step forward.[43, 44]

However, there are still big challenges for miRNA mimics and inhibitors, which are negatively charged and are difficult to bind to plasma membrane and enter the cell.[45] Moreover, improving delivery systems, optimizing the stability of miRNAs, and controlling off-target effects also remain hurdles for future development.

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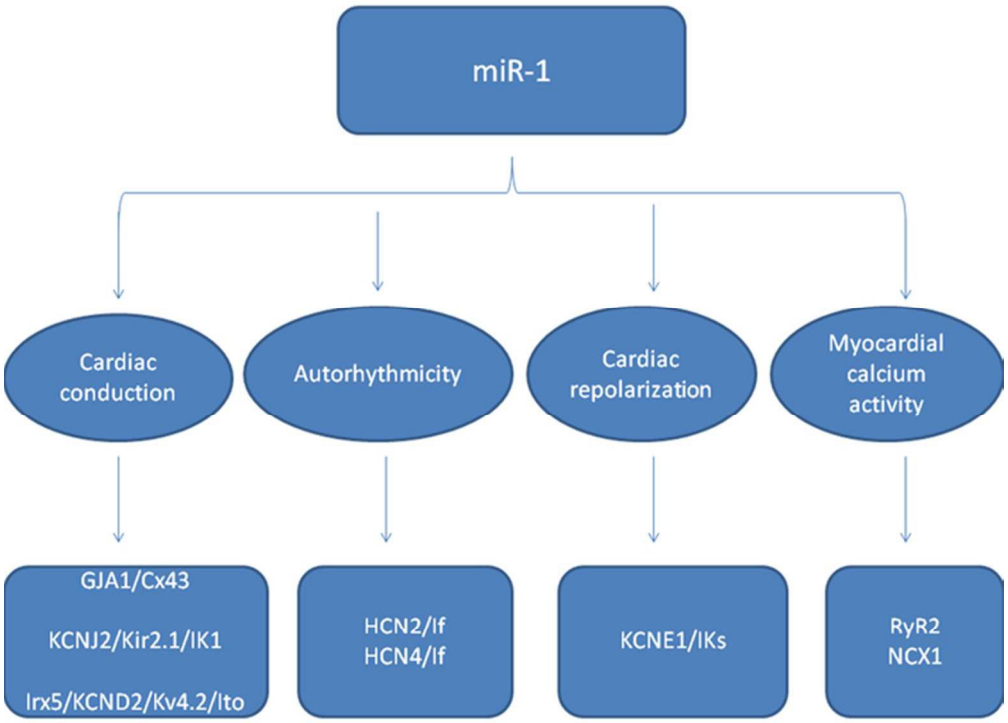
Figure 1: MiR-1 affects cardiac conduction, autorhythmicity, cardiac repolarization and myocardial calcium activity by regulating a variety of ion channels and proteins.

Figure 2: Biological principles of miRNA. MiRNA genes are transcribed by RNA polymerase II (Pol II) to generate the primary transcripts (Pri-miRNA). Drosha crops Pri-miRNA into precursor-miRNA (Pre-miRNA), which is exported from the nucleus to the cytoplasm by Exportin-5. The Pre-miRNA is cleaved by the Dicer in the cytoplasm. One strand of the duplex Pre-miRNA is taken into the RNA-induced silencing complex (RISC) and miRNA interacts with its target. It can induce translational repression when miRNA imperfectly bind to its mRNA target and mRNA cleavage when matching is perfect.

Figure 3 Alignment of has-miR-1-1 and hsa-miR-1-2 of miR-1 in human. Hsa-miR-1-1 and hsa-miR-1-2 are located at chromosomal regions 20q13.33 and 18q11.2, respectively. The two different precursors have the same mature form of miR-1. The mature miR-1 sequence is with red font.



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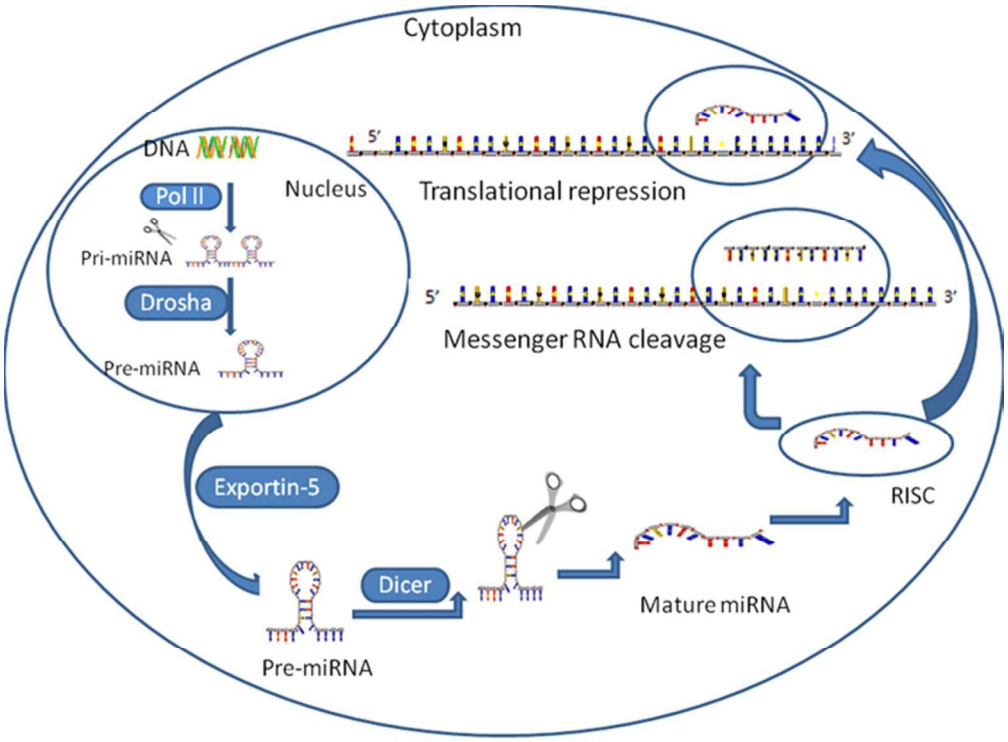

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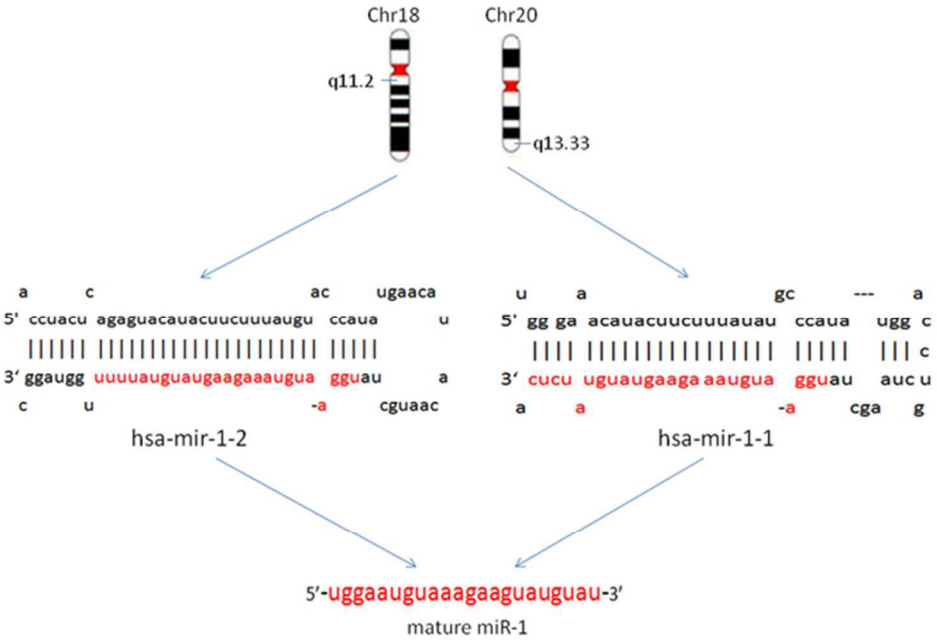

59x44mm (300 x 300 DPI)