Funct Integr Genomics
DOI 10.1007/s10142-013-0344-1

REVIEW

Human RNAi pathway: crosstalk with organelles and cells

Sadegh Azimzadeh Jamalkandi · Esmaeel Azadian · Ali Masoudi-Nejad

Received: 6 May 2013 / Revised: 3 October 2013 / Accepted: 7 October 2013

Abstract Understanding gene regulation mechanisms has been a serious challenge in biology. As a novel mechanism, small non-coding RNAs are an alternative means of gene regulation in a specific and efficient manner. There are growing reports on regulatory roles of these RNAs including transcriptional gene silencing/activation and post-transcriptional gene silencing events. Also, there are several known small non-coding RNAs which all work through RNA interference pathway. Interestingly, these small RNAs are secreted from cells toward targeted cells presenting new communication approach in cell–cell or cell–organ signal transduction. In fact, understanding cellular and molecular basis of these pathways will strongly improve developing targeted therapies and potent and specific regulatory tools. This study will review some of the most recent findings in this subject and will introduce a super-pathway RNA interference-based small RNA silencing network.

Keyword RNAi · piRNA · Integrated pathway · Silencing pathways · Small RNA · Non-coding RNA

Sadegh Azimzadeh Jamalkandi and Esmaeel Azadian contributed equally to the manuscript.

Electronic supplementary material The online version of this article (doi:10.1007/s10142-013-0344-1) contains supplementary material, which is available to authorized users.

S. Azimzadeh Jamalkandi
Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

E. Azadian · A. Masoudi-Nejad (✉)
Laboratory of Systems Biology and Bioinformatics (LBB), Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
e-mail: amasoudin@ibb.ut.ac.ir
URL: http://LBB.ut.ac.ir

Published online: 07 November 2013

Introduction

RNA interference (RNAi) is a highly conserved pathway responsible for transcriptional and post-transcriptional regulation of gene expression and triggered through double strand RNA (dsRNA) and small RNAs (Azimzadeh Jamalkandi and Masoudi-Nejad 2009, 2011). MicroRNAs (miRNAs), small interference RNAs (siRNAs), and piwi-interacting RNAs (piRNAs) are all examples of these small RNAs that comprise a variety of functions from gene expression regulation to viral defense and also transposon inactivation. Each of these pathways has critical roles in human physiology and disease, and these are mentioned in more detail in the sections below.

The pathway itself is derived from two major steps which are controlled by two core proteins. During the first key step, Dicer protein (Endoribonuclease) recognizes dsRNAs as its substrate and generates small RNAs. In the second step, Argonaut (Ago) takes the small RNAs and screens complementary target mRNAs. Target mRNA will be degraded or its translation will be suppressed during a process called post-transcriptional gene silencing (PTGS). DsRNAs can be generated from different pathways and with different names. MiRNAs are transcribed from host or viral genome and piRNAs are generated from germline cell's genome, while siRNAs are generated during viral replication that produces dsRNA. Each pathway will be examined separately, and their crosstalk, or interchange, will be assessed in order to understand the complexity of the pathway.

MiRNA pathway

miRNA origination and classification

Within the human genome, miRNAs spatio-temporally modulate gene expression. They are transcribed from various

regions (intergenic and intragenic) by RNA polymerases II/III (Zhou et al. 2008; Corcoran et al. 2009; Monteys et al. 2010). Usually, intergenic miRNAs have their own expression system including promoter and terminators and are located between protein coding genes. But, about one third of miRNAs are located within the introns of protein coding and non-coding sequences and are transcribed from their host promoter (intragenic miRNAs or mirtrons).

Besides their complex classification, these pathways are divided into two main pathways, including canonical and non-canonical pathways. The former is started by biogenesis of special stem-loop structure called primary miRNA (pri-miRNA) which should be processed to precursor miRNA (pre-miRNA) (Fig. 1). The latter joins to the canonical pathway from various branches (Fig. 2). For example, mirtrons (short intronic microRNA) (Parsi et al. 2012; Gokhale et al. 2012; Yan et al. 2011) and simtrons (splicing-independent mirtron-like miRNAs) (Havens et al. 2012; Curtis et al. 2012), shRNAs (short hairpin RNA) (Mueller et al. 2012; Dallas et al. 2012), snoRNAs (small nucleolar RNAs) (Scott and Ono 2011; Ono et al. 2011), and tRNAs (Schopman et al. 2010; Lee et al. 2009) which will be discussed in more details may join as alternative pathways to pri-miRNA/pre-miRNA steps (Fig. 1).

The different sources and mechanisms of miRNA generation show their high flexibility of complex regulatory functions. Additionally, a single miRNA can hit several targets or several miRNAs may target a single mRNA (Bartel 2004). In the first case, miRNA is probably a critical regulatory element which controls several targets in various conditions or even simultaneously. But in the second case, the target mRNA is a critical transcript that should be controlled in different conditions or its tight regulation is critical (Perron and Provost 2009).

MiRNAs have a variety of roles in normal cell growth and developments including apoptosis, development, cell proliferation, and signaling. In addition to these normal functions, they are implicated in many diseases such as cancer and hepatitis. Therefore, among all small RNAs, miRNAs are a special case which, besides gene expression control, are involved in cell–cell and cell–organelle signaling phenomenon (Ro et al. 2013; Lung et al. 2006).

Interestingly, some miRNAs are located within nucleus and can bind to other pre-miRNAs and inhibit its downstream processing (Tang et al. 2012). This means that miRNAs can control another miRNA's biogenesis in the nucleus (Fig. 2).

Canonical miRNA pathway

While transcribing microRNA genes (MIR genes), the transcript starts to fold to a hairpin like secondary structure called pri-miRNA. This long stem loop motif is specified by a terminal loop and single stranded RNA extension at both 3′ and 5′ ends which are critical for an enzymatic complex called microprocessor complex (Fig. 1). In fact, two large and small complexes bind to pri-miRNAs. The large complex is comprised of RNA processing and RNA transportation proteins (Gregory et al. 2004; Wada et al. 2012), but the small one is critical for pri-miRNA processing and is called microprocessor complex.

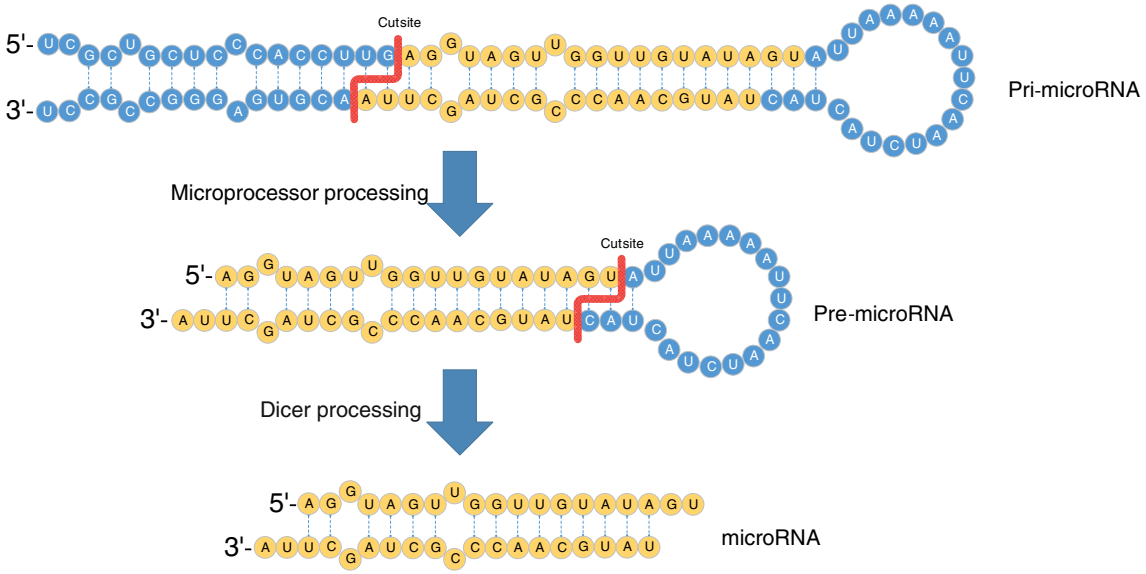

Fig. 1 miRNA generation. Pri-microRNA is cleaved through microprocessor complex including Drosha and DGCR8. The resulted molecule is called pre-miRNA which has 3′-2 nt overhangs. After transporting via Exportin-5, pre-miRNA will be recognized with Dicer, a ribonuclease, and will be cleaved to about 21 nt molecules called mature miRNAs. These miRNAs are proper for Ago protein and target mRNA finding and induction of PTGS

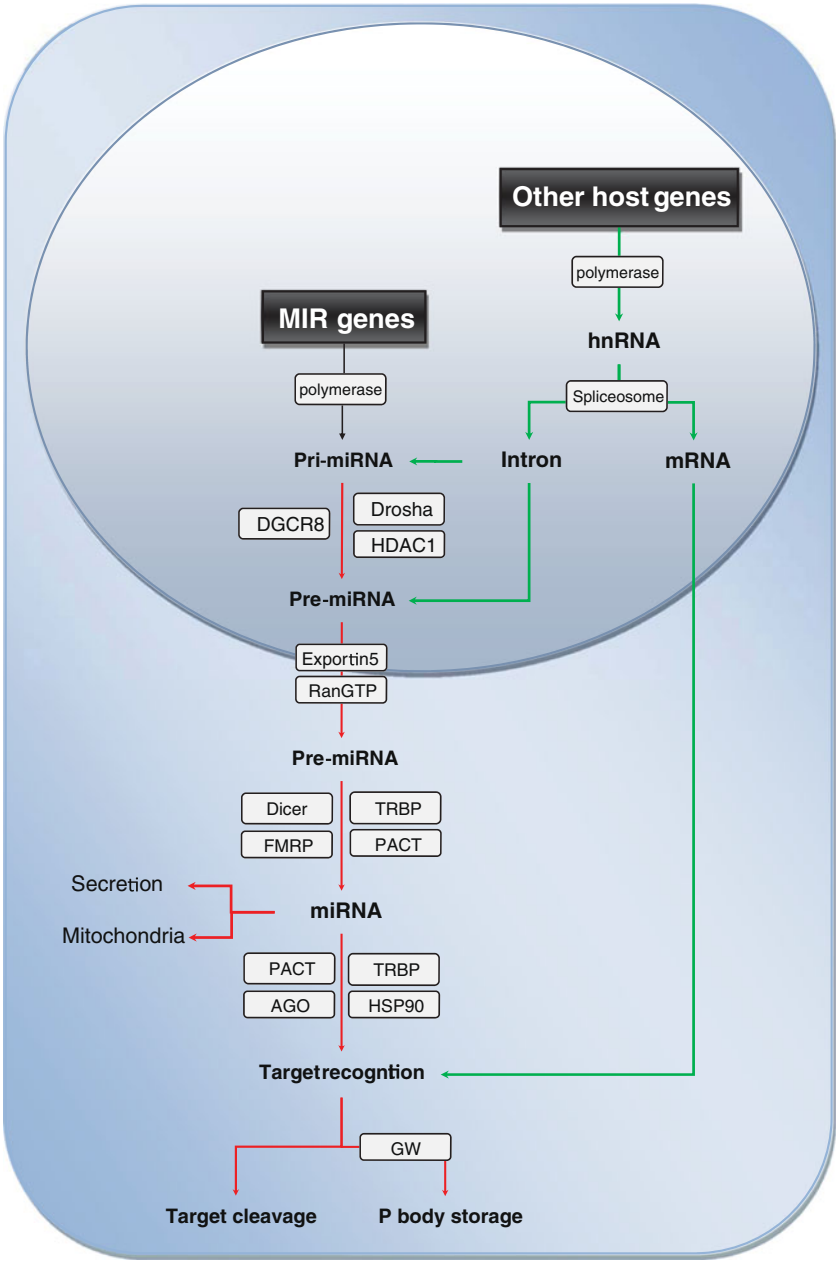

Fig. 2 MiRNA generation and its mechanism of action. Different originations, different regulatory levels, and different target locations are illustrated

The microprocessor complex recognizes, binds to, and cleaves pri-miRNA and then releases an ~60–70-nt-long stem loop fragment called pre-miRNA in which mature miRNA/miRNAs is buried within its arms. Drosha (RNase III family nuclease), DGCR8 (DiGeorge critical region 8: a double-cysteine-ligated Fe (III) heme protein), and HDAC1 (histone deacetylase 1) are the main components of microprocessor complex. DGCR8 is a highly stable protein and has two dsRNA-binding domains that achieve the best conformation for dsRNA by heme binding (Barr et al. 2011, 2012). The heme-bound DGCR8 is more active than its free form which recognizes pri-miRNAs and then binds to Drosha. Also, it has also been reported that HDAC1 binds to, deacetylates, and increases the affinity of DGCR8 to pri-miRNA (Wada et al. 2012). In fact, it indirectly increases the rate of miRNA generation. In general, HDAC1 is involved in transcriptional suppression of gene expression via promoter histone deacetylation and increasing miRNA production by increasing DGCR8 affinity, respectively (Wada et al. 2012).

Drosha is responsible for recognizing and polishing pri-miRNAs by cleaving it with special structure. This binding is called a conserved structural functionality since no conserved sequences are found on pre-miRNA for Drosha (Ohrt et al. 2006). Furthermore, Drosha binds and cleaves various pri-miRNAs in different efficiencies and specificities. As reports show, conserved substrates are processed more efficiently (Feng et al. 2011). Besides regulatory effects of heme and HDAC1, there are two hairpin regions at 5′ UTR and one at

of DGDR8 transcript (pre-miRNA like structure) that is a cleavage site for microprocessor complex (Triboulet et al. 2009; Han et al. 2009). This strategy prevents DGCR8 accumulation and its non-specific binding (Triboulet et al. 2009; Shenoy and Blelloch 2009). Interestingly, caspases cleave DGCR8 at heme-binding domain to stop miRNA generation in apoptotic cells (Gong et al. 2012). This may describe the low level of miRNA concentrations within the apoptotic cells. In summary, DGCR8 is a regulatory intermediate in the microprocessor complex. In the trimerization model, a higher-order structure of trimerized DGCR8 cooperatively recognizes one or two pri-miRNAs (Faller et al. 2010). This composition can prepare the substrate profit for Drosha binding and cleavage. Then, bring in mind that these events happen co-transcriptionally (Shenoy and Blelloch 2009) (Fig. 2). Surprisingly, it has been reported that in some cells, DGCR8 promoter is under regulation of ING1 (inhibitor of growth) (Gomez-Cabello et al. 2010). This tumor suppressor protein regulates diverse pathways such as cell cycle and apoptosis. It can recognize histone marks and recruit histone modifiers to the targeted promoters (Gomez-Cabello et al. 2010). Then, it is environmentally controlled by heme, transcriptionally by ING1, and post-transcriptionally by HDAC1 and its insider miRNAs.

Shipping pre-miRNA to cytoplasm

After microprocessor cleavage, released pre-miRNA has 2 nt 3′ overhang which is a motif recognized by Exportin-5 in the nuclear membrane and then Dicer in the cytoplasm. The 3′ overhang and the length of stem (>16 bp) are critical for this binding (Zeng and Cullen 2004). Exportin-5 was first reported as a dsRNA karyopherin (proteins involved in cytoplasm–nucleus transportation in eukaryotic cell) (Brownawell and Macara 2002) that can specifically bind to RanGTP (GTP-binding nuclear protein Ran (RAs-related nuclear protein)) which interact with nucleoporins and then shuttles between the nucleus and cytoplasm. Exportins need RanGTP proteins for efficient loading and export of RNAs (Calado et al. 2002) (Figs. 2 and 5). This binding is structure dependent and sequence independent (Bohnsack et al. 2004). So, it may export host pre-miRNAs or viral hairpin RNAs and some tRNAs (Chen et al. 2004) or shRNAs with structural similarity to pre-miRNAs (Yi et al. 2005). In addition, ILF-3 (interleukin enhancer binding factor), a dsRNA binding protein, interacts with Expotrin-5 in a RanGTP depending way (Brownawell and Macara 2002; Yi et al. 2003). ILF-3, Exportin-5, RanGTP, and pre-miRNAs are assembled together in a quaternary complex in which pre-miRNA makes an interaction bridge between Exportin-5 and ILF-3 (Gwizdek et al. 2004). This quaternary complex besides exporting pre-miRNA keeps it preserved of exonucleases. Interestingly, Exportin-5 transports Dicer mRNA to cytoplasm and regulates Dicer expression. Inhibition of exportin-5 also downregulates Dicer expression (Bennasser et al. 2011). Also, Exportin-5 saturation by pre-miRNA overload causes export loss of Dicer mRNA (Bennasser et al. 2011). Interestingly, viral-associated RNA-1 (VA1 RNA) of adenoviruses overexpression saturates Exportin-5 and therefore decreased cellular pre-miRNA export competitively but with no effect on siRNA basal silencing power (Perron and Provost 2009).

Pre-miRNA arrives in the cytoplasm

After the directional transportation of pre-miRNA toward the cytoplasm, dicer takes it. This protein is crucial for organism survival indicating deep functions of miRNAs and other dicer dependent small RNAs in differentiation and development of the cells (Perron and Provost 2009; Koscianska et al. 2011). This enzyme catalyzes small RNA excision from various substrates including long dsRNAs and pre-miRNAs. Dicer fidelity and length specificity and processing several different substrates are achieved by its various domains and cofactors (Ma et al. 2012). Size of Dicer-diced products depends on its PAZ and RNase III domains on Dicer (Ma et al. 2012) (Fig. 2). Furthermore, more interestingly, Dicer can discriminate poor functional substrates from highly functional by 2 nt 3′ overhangs and thermodynamic properties (Sakurai et al. 2011). So, Dicer has a role in pre-selection of active siRNAs.

PACT (protein kinase R-activating protein; a protein activator of the interferon-induced protein kinase) and TRBP (HIV-1 TAR RNA-binding protein) directly interact and then bind to Dicer and make a triplex complex (Kok et al. 2007). Dicer-TRBP electron microscopy (20 Å resolution) revealed L-shaped interaction that prepares a putative site for dsRNA binding (Lau et al. 2009). Dicer and TRBP cut pre-miRNA to miRNA duplex with 2 nt 3′ overhangs (Perron and Provost 2009). TRBP has interactions in RNA modifications, processing, and with several transcription factors (Chi et al. 2011). TRBP can directly bind to Dicer between its ATPase and helicase domains probably through its C4 domain (Daniels et al. 2009). Dicer is much faster in the presence of TRBP on both miRNA and siRNA substrates. Moreover, TRBP increases Dicer-substrate stability (Chakravarthy et al. 2010).

The characteristics by which Dicer recognizes its targets are in summary (Park et al. 2011):

- 3′ anchoring and counting: measuring the specific distance from 3′
- 5′ anchoring and counting
- Terminal loop which is important for Dicer processing (Zhang and Zeng 2010)
- 5′ pocket in Dicer that recognizes 5′ phosphate

- dsRNA binding proteins such as TRBP, PACT, and RBM3 (putative RNA-binding protein 3)

PACT and TRBP have strong homology (44 % similarity) and make heterodimers localized in specific areas within the cytoplasm in the absence of dsRNAs (Perron and Provost 2009; Laraki et al. 2008). PACT and TRBP are both dsRNA-binding proteins that can also bind to interferon-induced protein kinase R (PKR). PKR is a critical component in the switching on/off interferon antiviral defense pathway. Basically, in the absence of dsRNA molecules, the TRBP–PACT heterodimer cannot activate PKR but dsRNA (minimal length of 30 bp) or PACT homodimes can both bind to PKR and induce its dimerization. Subsequently, PKR is auto-phosphorylated and activated (Sarkar and Sen 2004; Lemaire et al. 2008). Eventually, activated PKR can phosphorylate eIF2a (alpha subunit of eukaryotic initiation factor 2) and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) further inducing translation arrest but interferon expression, respectively. Stopping protein synthesis in infected cells and local spreading viral infection signal are two main consequences of PKR activation. Apoptosis induction is also reported through caspase 8. In summary, however TRBP inhibits PKR activation but PACT induces it (Laraki et al. 2008). This is the exciting story of RNAi viral control and signaling in mammals which is able to induced interferon signaling and cell death in response to long dsRNAs recognition. Interestingly, during stresses PACT is phosphorylated and then binds to PKR to activate it. PACT-p has a weak affinity to bind to TRBP which can escape from TRBP and bind to PKR (Singh et al. 2011). Then, generated miRNAs should be delivered to another complex called RISC (RNA induced silencing complex) (Fig. 2).

RISC takes mature miRNA and finds target mRNA

After first cleavage, Dicer hands off small RNA to RISC and starts next cleavage (Ando et al. 2011). Dicer repositioning on the dsRNA following the dicing helps in directional and orientated delivery of the products to RISC (Noland et al. 2011). RISC selects one strand of the small RNA. Ago, PACT, TRBP, and siRNA/miRNA are core components of RISC. Ago-2 forms the catalytic complex of silencing (Chendrimada et al. 2005), and TRBP is necessary for its recruitment and physical association to Dicer–TRBP (TAR RNA-binding protein). Ago-2 directly received double stranded-miRNA and then cleaves the passenger strand (Matranga et al. 2005). Dicer–TRBP prepares a platform for Ago-2 recruitment and loading and therefore RISC assembly (Chendrimada et al. 2005). Target site features are critical for Ago selection and binding (Hausser et al. 2009). Also, poly-A tail length is important for Ago function (Iwasaki and Tomari 2009). There is a report on Ago-3 involvement in translational inhibition and Ago-2 in cleavage of the targets, most (Omel'ianchuk et al. 2011).

Loading of siRNA/miRNA into RISC activates it as it can bind target mRNAs mostly at their 3′ UTR (Wightman et al. 1993; Lee et al. 1993). Many factors affect miRNA specificity and activity including seed sequence, G:U wobble, and pivot nucleotide (Brodersen and Voinnet 2009; Brennecke et al. 2005; Doench and Sharp 2004; Stefani and Slack 2012). After RISC binding to target mRNA, target cleavage, or target translation, repression will happen. The former is irreversible phenomenon (Meister et al. 2004; Liu et al. 2004), but the latter is reversible (Maroney et al. 2006). In fact, cleavage is a very secure method, but reversibility of silencing could be more important which indicates flexibility of the system during target degradation. During activated Ago binds and recruits poly-A removal, and exosomes with 3′ to 5′ exonuclease activity degrade tailless mRNAs. It is not fully understood how Ago-2 is inhibited from cleaving, but imperfect matching/bulge formation may regulate slicer activity of it.

P-bodies (processing body) (Franks and Lykke-Andersen 2008) are located in the cytoplasm of yeast to human. These dynamic bodies respond to temperature, osmotic pressure, glucose level, and also infection (Kedersha et al. 1999). Reversible mRNA silencing may happen in these bodies. These miRNA–mRNA–RISC complexes are transported to p-bodies or transported back towards the cytoplasm.

As mentioned above, Ago proteins are one of the critical proteins in target cleavage. Among eight human Ago proteins, just four of them are expressed in the vast majority of the cells (Meister et al. 2004). Ago-1–4 proteins are core components of RNAi machinery in which only Ago-2 has slicer activity but others can just load miRNAs. Ago-2, Ago-1, and Ago-3 interact with 60, 30, and 10 % of cellular miRNAs, respectively. Shotgun proteomics data indicate the similar abundance of these proteins within cells (Valdmanis et al. 2012). Ago-2 that has the highest expression rate can cleave target mRNA, but all Ago proteins can role in non-cleavage-mediated inhibition. The function of other Ago proteins remains uncertain.

Ago-2 and Ago-3 are mostly immunopurified with miRNAs, and Ago-3 is localized in P-bodies without cleavage activity (Azuma-Mukai et al. 2008). All four Ago proteins contribute to miRNA pathway (Su et al. 2009). Moreover, crystal structures show that Ago-2 is a bilobed molecule and can bind to guide miRNA (nucleotides 2 to 6) and target mRNA via a central cleft (Schirle and MacRae 2012). N domain of Ago-2 initiates the unwinding and assembly of miRNA duplex into the RISC (Kwak and Tomari 2012). Loading miRNA duplex, unwinding, and removing passenger strands are three steps for mature and active RISC formation. Binding to miRNA increases Ago stability and locks its flexibility in a specific conformation (Elkayam et al. 2012). Also Ago-2 is physically bond to Dicer–TRBP critical for

RISK assembly (Chendrimada et al. 2005). It is stated that heat shock protein 90 (HSP90) is also involved in Ago regulation.

Accordingly, HSP90 is necessary for free Ago stability (without RNA) as its inhibition results Ago destabilization (Johnston et al. 2010; Suzuki et al. 2009). This protein is effective in stable interaction of Dicer and Ago-2 for miRNA hand-off (Tahbaz et al. 2004). Inhibiting HSP90 resulting to Ago and GW182 decrease and consequently resulting to P-bodies size decrease may be due to GW182 instability (Suzuki et al. 2009; Pare et al. 2009). Furthermore, HSP90 has more affinity to free Ago-2 than its activated form indicating its function on efficient loading of miRNA on Ago-2 (Johnston et al. 2010).

Ago-2 can recruit GW182 that has three Ago-2 binding sites (Takimoto et al. 2009; Han et al. 2004). Expression of GW182 and Ago-2 is upregulated after siRNA delivery (Jagannath and Wood 2009). These small molecules together with Ago-2 are rapidly localized to p-bodies, and then, Ago proteins are regulated by phosphorylation at specific residues as serine-387 phosphorylation mediated by p38 MAPK pathway which increases Ago-2 P-body localization (Rudel and Meister 2008; Rudel et al. 2011; Zeng et al. 2008). However, Ago proteins are distributed within the cytoplasm, but they accumulate within new bodies called stress granules. Also, after the stress miRNAs are necessary for P-body localization but not for stress granules (Detzer et al. 2011; Leung et al. 2006). Ago-1 and Ago-2 proteins are located and concentrated in the p-bodies (Sen and Blau 2005). Some reports states that Ago-2 is diffused in cytoplasm and nucleus (Rudel et al. 2008) and has also interactions with GW182 (known as P-body marker) (Ikeda et al. 2006).

Also, they are mRNA decay and deposition center, include mediators of mRNA transportation, degradation, and release decapping, deadenylation, and RNA degradation/repression enzymes which are induced here. Active Ago-1–4 localization have been shown in theses bodies. Actually, it is a functional site for mRNA turnover. Blocking these bodies by GW182 depletion impairs miRNA-based silencing. Also, there are possibility of RISC assembly and activation within these bodies. P-body can be sectioned into three parts including decapping, deadenylation, and nucleases. Also, Gem-associated protein 5 (Gemin5), RNA-associated protein 55 (Rap55), p54, and fragile X mental retardation protein (FMRP) are needed for mRNA storage and translational repression. Interestingly, what can control mRNA return into the cytoplasm is unknown. miRNAs are detected within P-bodies when co-precipitating Let-7 with GW182 (Perron and Provost 2009) (Fig. 2).

Non-canonical miRNAs

The microprocessor complex which was introduced earlier is not necessary for generation of all miRNAs. Mirtrons and simtrons (splicing-independent mirtron) are example of such pathways. Mirtrons are derived from introns in a splisosome-dependent and microprocessor-independent manner (Havens et al. 2012; Ruby et al. 2007). But, simtrons are derived from introns and are splisosome independent and microprocessor dependent (Havens et al. 2012). Interestingly, some like miR-451 are generated by Ago-2 prior to Dicer cleavage (Dallas et al. 2012; Yang et al. 2010). This molecule is highly conserved in vertebrates and is processed by Drosha as a short hairpin which can directly load onto Ago-2 (Yang et al. 2010). We may classify this as a simtrons that contributes in several pathways (Bandres et al. 2009; Tsuchiya et al. 2009; Cheloufi et al. 2010). Additionally, some mirtrons have their own promoters (polymerase II or III binding sequences) and some are transcribed from their host gene's promoter sequences (Monteys et al. 2010; Li et al. 2007). They may transcribe co-dependently expression with their host genes (Ma et al. 2011); miRNA-mimicking RNAs were also observed.

Viral miRNAs

Organisms tend to have tightly controlled expression systems, but viruses have acquired many interesting strategies to misuse the host expression system or to circumvent the defense system of the cell. One such strategy is the miRNA system (Skalsky et al. 2012; Wu et al. 2011). It is clear now that dsRNA viruses encode MIR genes (vi-miRNA) which make them capable to control viral or host expression system. No vi-miRNA is identified in RNA viruses yet (Meckes et al. 2010), but in Simian polyomavirus 40 (SV40), Epstein–Barr virus (EBV), human immunodeficiency virus (HIV-1), and Herpes simplex virus-1 (HSV-1) genome, they are identified. They can enter into the miRNA pathway from various branches; some bypass the microprocessor complex and some are derived from degraded viral tRNAs (Bogerd et al. 2010); some directly load into Dicer and some hijack the host microprocessor and effector complexes of silencing (Lin and Sullivan 2011). In some cases, there is a competition between host and vi-miRNA nuclear-2-cytoplasm export by Exportin-5 (Bennasser et al. 2011). Transiently some viRNAs can directly load into Ago-2 (Perron and Provost 2009) (Fig. 5). Generally, they can control apoptosis (Gupta et al. 2006), immune system (Stern-Ginossar et al. 2007), cancer, etc.

miRNA circulation

MiRNA horizontal transfer through blood circulation system and various body fluids (about 12 body fluids) is reported repeatedly in saliva (Courts and Madea 2011; Gallo et al. 2012; Michael et al. 2010; Park et al. 2009; Patel et al. 2011; Zhang et al. 2009), semen and urine (Gidlof et al. 2011; Hanke et al. 2010; Scian et al. 2011; von Brandenstein et al. 2012), amniotic fluids, tear, and milk (Iguchi et al. 2010; Fleischhacker and Schmidt 2007). Presence of mobile miRNAs

is also demonstrated in human plasma with a remarkable stability against RNase-A/T and RNase H enzymes (El-Hefnawy et al. 2004) and sensitivity against proteases. This means that circulating miRNA acquires a system by which it can escape from degradation. Intravascular transportation system (exosomes, apoptotic bodies, and microparticles) and extravesicular transport (protein complexes and lipoprotein complexes) are both observed. There are two facts about circulating miRNAs that should be answered: (1) do cells really produce circulating miRNAs or the reports are technically biased? And if yes, (2) do circulating miRNAs induce silencing signal in recipient cells?

It is known that some cells secrete miRNAs as cell–cell signaling molecules (intercellular/interorgan). Circulating miRNAs were separated in protein complexes (Ago-2 co-purified with miRNAs) more than in vesicles in the first study (Arroyo et al. 2011) (Fig. 5). Also, some researchers stated that there were technical limitations and Ago-stuck miRNAs were released from lysed cells (Mitchell et al. 2008). Eventually, a proficient research showed that human THP-1 cells selectively package miR-150 into active microvesicles in vitro. These secreted microvesicles are delivered specifically into human HMEC-1 cells and induce their migration as an inflammatory response (Zhang et al. 2010). Moreover, repeated experiments in vitro and in vivo indicated similar miRNA patterns, especially in disease and cancer (Zhang et al. 2010). Circulating miRNAs are actively and specifically secreted in response to different stimuli (Zhang et al. 2010). Eventually, secreted miRNAs could be called extracellular miRNAs or exogenous miRNA for recipient cells. In this landing, donor cells can be blood cells or any other tissue cells (Chen et al. 2008), but regulation of recipient cell expression is unclear. However, it is demonstrated that released miRNAs deliver precisely and regulate the targets genes specifically (Valadi et al. 2007). This means that miRNAs cells can interact with specific target cells. But, the boarding and landing of these miRNAs are not understood yet. The pathways that induce silencing are not understood yet, but in plants a signal intensification process called silencing amplification is used to produce secondary siRNAs (Azimzadeh Jamalkandi and Masoudi-Nejad 2009, 2011). HDL (high-density lipoprotein) also transfers and delivers miRNAs toward recipient cells (Regazzi and Widmann 2012; Zampetaki et al. 2012; Vickers et al. 2011). Delivered circulating miRNAs are functional on target cells, too (Vickers et al. 2011). Scavenger receptor BI may be necessary for delivery (Vickers et al. 2011), but how a single miRNA can spark regulatory action in the recipient cells in the case of small RNA amplification pathway is not reported in human. Direct binding of protein complexes also are reported for stable miRNA transportation. Circulating bodies such as exosomes interact with recipient cells using receptor–ligand interactions and then fuse to their membrane (Zampetaki et al. 2012; Cocucci et al. 2009).

Circulating miRNAs are nominated as fingerprints of various diseases and promising biomarkers (Zhou et al. 2012a; Zhao et al. 2012; Zen and Zhang 2012; Zahm et al. 2012; Yang et al. 2012; Wang et al. 2012a). Also, natural delivery of circulating miRNAs via physiological carriers is an encouraging method for improvement of specific drug delivery systems. So, there is huge commercial interest on these small molecules as an ideal biomarker attributing non-invasive, rapid, highly sensitive, and specific characteristics.

Transcriptional gene silencing vs. activation

RNAa

It was first believed that small RNAs especially miRNAs can only induce transcriptional and post-transcriptional silencing levels. During PTGS, unceasing gene expression occurs and just target mRNA is degraded, but in the case of epigenetic silencing (also called transcriptional gene silencing (TGS)) expression is thoroughly inhibited in a potent and long lasting method. Technically, both ways are available for scientists, but what about inducing or increasing transcription rate of target genes by a very similar method? Fascinatingly, it is identified that small RNAs can also induce expression of target genes. Many mature miRNAs translocate back to the nucleus for TGS or activation-targeted promoter sequences (Liao et al. 2010; Younger et al. 2009; Kim et al. 2008).

RNA activation (RNAa) is a process that small dsRNAs (called saRNA) complementary to promoter region can upregulate gene expression in *cis*, nominating it as a new tool for gene overexpression studies (Wang et al. 2012b). This novel mechanism is reported in several organisms including mammals (Huang et al. 2010). Some human saRNAs are functional in other organisms indicating conserved RNAa pathway. Therefore, small RNAs especially miRNA may induce (RNAa) or inhibit (RNAi) gene expression (Place et al. 2008; Li et al. 2006; Janowski et al. 2007). It is clear now that RNAa utilizes epigenetic modifications during transcriptional activation (Portnoy et al. 2011; Place et al. 2010). Discriminately, RNAi and RNAa kinetics are distinct, but both are Ago-2 dependent. Universally, Ago-1 and Ago-2 downregulations decreased RNAa via promoter interaction (Li et al. 2006; Kim et al. 2006; Janowski et al. 2006; Chu et al. 2010). Ago-1 and Ago-2 are abundant in the nucleus as Ago-1 is placed on proximal *cis* sequences interacting with RNA pol II and histone H3 trimethyl Lys4 (H3K4me3) (Huang et al. 2012). Also, HDAC1 may be recruited to the activation complex at the promoter region. There are evidences that pol II and Ago-1,2 are required (Kim et al. 2006; Janowski et al. 2006; Weinberg et al. 2006). The transcript which has 3′ UTR overlapping with promoter region is also required for siRNA-directed silencing (Han et al. 2007). In neuron-specific genes,

there is a motif called neuron-restrictive silencer element (NRSE) which small dsRNAs including this sequence can trigger NRSE gene expression. This sequence is also recognized by special transcription factors (NRSF/REST), too. This emphasizes that a specific interaction between dsRNA, proteins, and DNA is required (Kuwabara et al. 2005). Furthermore, histone H3 Lys9 (H3K9) methylation is associated with Ago-2 and miRNA in the nucleus, confirming that epigenetic modifications can be inherited during proliferation (Li et al. 2006). Therefore, RNAa is correlated to histone modification, too.

Within this sequence-specific puzzle, there should be other components matching miRNAs to genome and screening it, and then identifying and modifying the target site. RNAa can stably induce gene expression as its 5′ end of seed sequence which is critical for active binding and silencing. As target sequence cleavage is not reported in RNAa phenomenon, Ago-2 may react with complementary DNA sequence in a similar way by targeting promoter sequences. However, the miRNA target recognition is very similar to RNAi-based mRNA target recognition roles (matches and mismatches) (Doench and Sharp 2004; Place et al. 2008). However, it is not clear as to why some promoters are resistant against RNAa, yet (Li et al. 2006).

In summary, RNAa is like RNAi, reproducible, specific, and potent. Actually, Ago should play an RNA-dependent DNA-binding role in RNAa unlike what is seen during RNAi. Also, there are many transcribing small RNAs from genome which are promoter components (Younger and Corey 2009). Linking sequence specific silencing or activation needs:

- Special *cis* elements on DNA;
- A component that recognizes a specific sequence (siRNA, miRNA and a transcription factor) and binds to;
- A small dsRNA binding protein (Ago-2);
- Other proteins (for DNA or histone modification, polymerization, modification maintenance). During transcription activation, RNA polymerase should be recruited, but in silencing methylation-related enzymes should be recruited.

Usually, omitting CpG islands and inverted repeat sequences from the upstream of the specific genes and then utilizing siRNA-directed silencing boasted expression about 2–10 folds. RNAa lasting for more days than RNAi may be due to epignenetic modifications and their inheritance (Morris et al. 2004). Also, IFN response had not been activated indicating a specific activation mechanism (Morris et al. 2004).

PiRNAs

Inactivating RNAi machinery will result in hypersensitivity against viruses. Otherwise, non-specific activation of RNAi using long dsRNAs activates interferon responses which cause general cellular translation inhibition in vertebrates (Fig. 4). Among animals, two distinct classes of Ago and piwi proteins exist that can bind to miRNAs/siRNAs and piRNAs, respectively. In germlines, transposable elements (TE) can be a source of genetic instability, as about 45 % of our genome is TEs. Interestingly, human germ cells have a plan for suppressing TEs. PiRNAs are produced in germ cells and are responsible for TE suppression (Aravin et al. 2007a) (Fig. 3). PiRNAs are the longest known small RNAs (24–31 nt) which are Dicer independent and highly abundant in germ cells responsible for TE suppression (Svoboda et al. 2004; Watanabe et al. 2006). They are processed by piwi sub-family, and some of them are involved in heterochromatin remodeling or RNA destabilization (Okamura and Lai 2008). They were first reported in fly and are germline-specific and endogenous small RNA (Aravin et al. 2001, 2003). When responding to TEs, they may be called repeat associated small RNAs (rasiRNA), too. They originate from intergenic repetitive elements or piRNA clusters (Aravin et al. 2006). They work independent from Dicer and Drosha and role through ping-pong cycling.

Moreover, two classes of piRNAs are reported up to now: pre-pachytene piRNAs and pachytene piRNAs (Watanabe et al. 2006; Aravin et al. 2006). Pre-pachytene piRNAs derive from TEs, but pachytene piRNAs originate from genomic clusters. In our body, H3K9 is methylated in the centromeric area (Rosenfeld et al. 2009) and is shown that centromere and kinetokore integrity is critically affected by Dicer-related RNAs (Chueh et al. 2009; Fukagawa et al. 2004). SiRNA can induce methylation of DNA and H3K9 (Hawkins et al. 2009). piRNAs are critical for spermatogenesis (Gao and Frohman 2012; Huang et al. 2011; Gu et al. 2010), TE suppression and silencing, epigenetic regulation (Sigurdsson et al. 2012), regulating mRNA stability, and translation (Aravin et al. 2007b). During spermatogenesis, piRNAs are associated with processing proteins and template RNAs in a region close to mitochondria called intermitochondrial cement in drosophila (Gao and Frohman 2012). PiRNAs are found in intergenic and intragenic genomic regions including introns and exons (Esposito et al. 2011). Interestingly, they are not restricted only to germ cells. They are reported in the somatic cells too (Esposito et al. 2011).

Transcriptional gene silencing

Besides dsRNAs which can make specific genes off via PTGS in cytoplasm, promoter complement RNAs (transcription start sites or more upstream sequences) can make gene expression silence by associating to target DNA sequences, too (Janowski et al. 2006). Small RNAs especially control epigenetic modifications in the genome. Among these small RNAs,

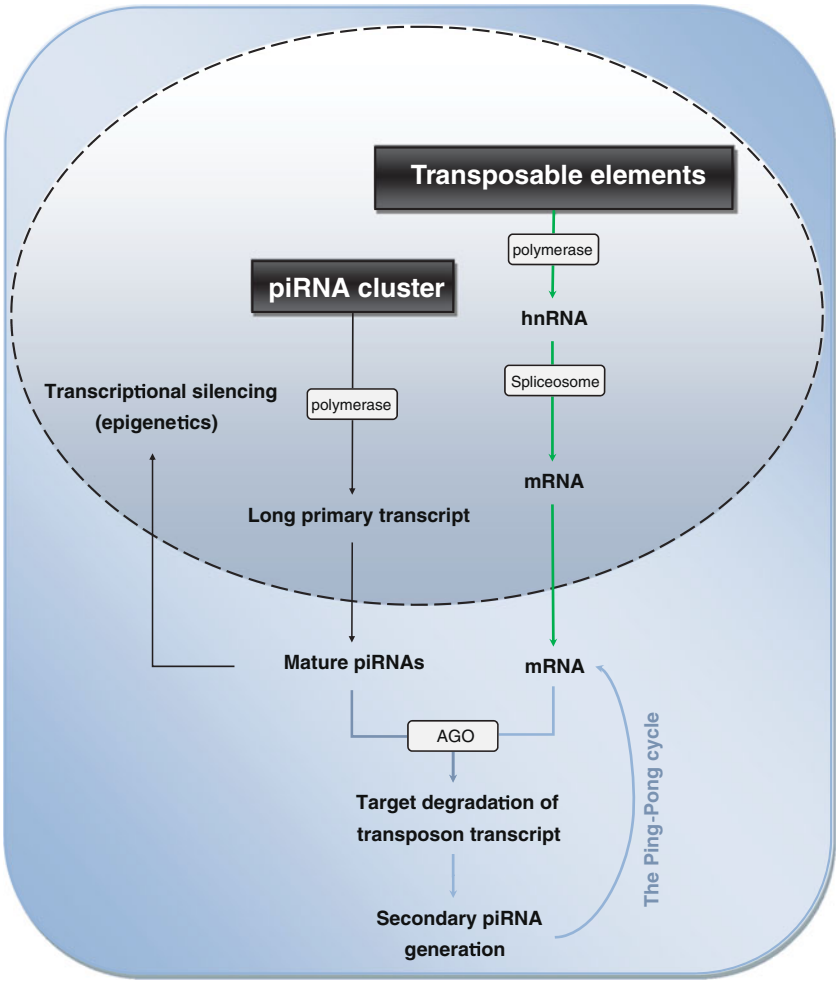

Fig. 3 piRNA pathway generates primary and secondary piRNAs

methylation of miRNA genes is a process of their transcription tuning and regulation (Wong et al. 2012; Yu et al. 2012; Wee et al. 2012; Minor et al. 2012). During TGS in human cells, Ago-1 binds to RNA polymerase II and initiates dimethylation of histone H3 Lys9 and heterochromatin formation. Other components including Polycomb protein and TAR RNA-binding protein-2 (TRBP2) co-localize with the mentioned components (Kim et al. 2006).

Cancer studies looking for genetic and epigenetics of deficient pathways have proved that there are many epigenetic modifications due to RNAi pathway such as hemoglobin A2 (HBA2) methylation comparing with normal cells (Pauler et al. 2007). Some other reports have shown that the RNA-directed gene silencing is also possible in mammals beyond plants, fly, and nematodes. In fact, CpG dinucleotide islands are presented within promoter sequences in which their dense methylation (hypermethylation) can make them silent mostly observed in cancer cells. It is also reported that small RNAs containing 5′-CG-3′ dinucleotide and 5′-CNG-3′ trinucleotide can induce promoter methylation. These small RNAs can inactivate several genes containing similar promoter sequences simultaneously (Galitskii 2008). Hypermethylated CpG islands within the regulatory sequences result in silencing.

There are systems for maintenance of the silencing, too. RISC, RNA machinery of TGS, can silence both the host genes and viral integrated genes (such as retroviruses). Successful promoter-targeted TGS is repeatedly reported (Zhou et al. 2012b; Turner et al. 2012; Chu et al. 2012), and some reports have stated the necessity of small RNA existence for silencing maintenance (Mohammad et al. 2012). Promoter targeting induces long-term TGS via histone methylation and DNA methylation for 3 days. Maintenance of the silencing requires Ago-1, DNA methyltransferase 3A (DNMT3a), and HDAC1 (Hawkins et al. 2009). During TGS, siRNA treatment increases H3K9 and H3K27 methylation at target promoters which depends on nuclear delivery of saRNAs. Just antisense strand of siRNA (guide strand) is functional requiring active RNA pol II transcription activity, histone methylation. The antisense strand of siRNA hybridizes to antisense strand of target promoter sequences (Weinberg et al. 2006; Han et al. 2007). Central mismatches do not avoid silencing process unless presented in both strands (Chu et al. 2012). TGS of some genes

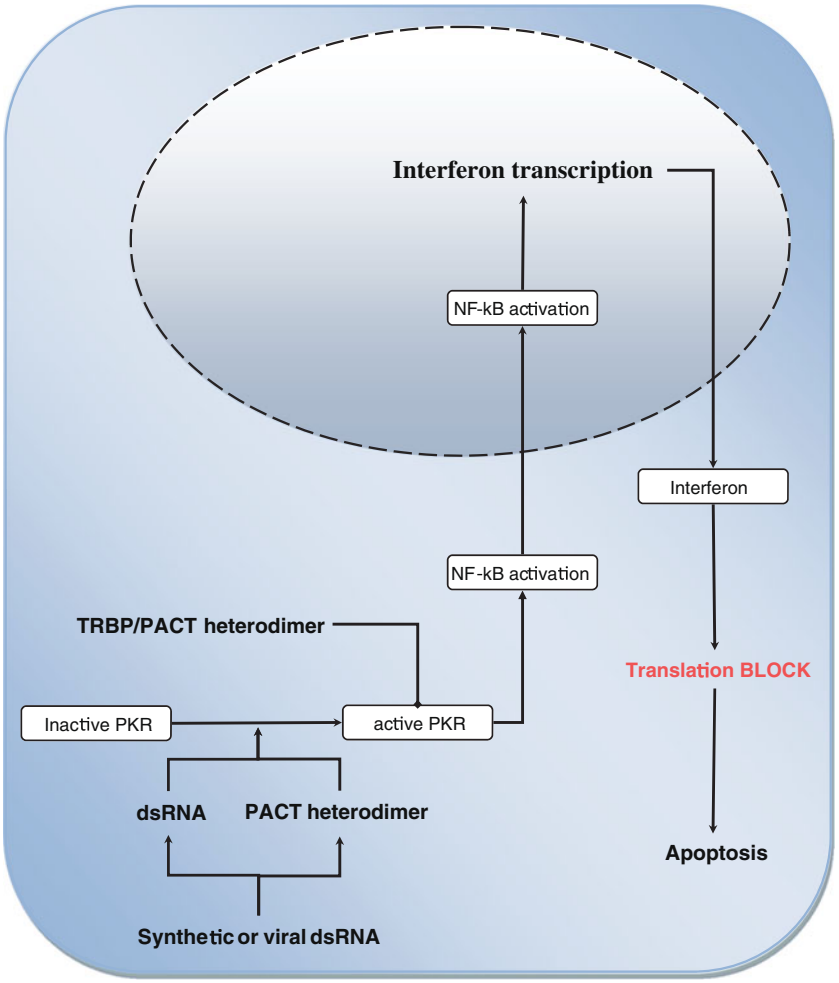

Fig. 4 Immune-based general silencing response

is reported that is in correlation with Dicer activity, as in Dicer mutants reactivating gene expression (Galitskii 2008; Ting et al. 2008). Ago-1 is co-localized with targeted siRNAs against Simian immunodeficiency virus (SIV) and HIV-1 infection, but Ago-2 is co-localized in the inner nuclear envelope as a novel translocation behavior (Ahlenstiel et al. 2012; Younger and Corey 2011). Ago-1 is distinguished in TGS associating with RNA pol II, histone H3 Lys9 dimethylation, Polycomb, and TRBP2 in silencing human immunodeficiency virus-1 co-receptor CCR5 and tumor suppressor RASSF1A along with siRNA-targeted Ras association domain-containing protein 1 (RASSF1A) promoter (Kim et al. 2006).

Tumor suppressor miRNAs are mostly hypermethylated and inactivated (Kozaki and Inazawa 2012; Wang et al. 2011). MiRNAs can contribute in DNA methylation by modulating DNA methylation machinery (Pan et al. 2010). Promoter-associated small RNA + Ago-1 + DNMT3a + HDAC1 are critical for transcriptional silencing, and DNMT1 is required for silencing maintenance (Weinberg et al. 2006; Hawkins et al. 2009). Ago-1 is introduced as major effector component of this process (Kim et al. 2006). Ago-1 and RNA polymerase II association is requisite for histone H3 Lys9 dimethylation and TGS induction (Kim et al. 2006). During senescence Ago-2, retinoblastoma-1 (RB1) and miRNAs physically and functionally interact to transcriptionally silencing of the target gene. Ago-2 directs miRNA toward the target (Benhamed et al. 2012).

Non-specific general silencing

During specific delivery of dsRNAs, non-specific silencing was also observed indicating a general regulatory pathway controlling dsRNA entrances in mammals. DsRNA induced interferon and inflammatory cytokines. Early works indicated that more than 30-bp-long dsRNAs induce the immune system, but later it was observed that some smaller dsRNAs can induce it, too. The secretome analysis shows that after dsRNA sensing in the cytoplasm, exosomal and lysosomal proteins are secreted as inflammasome activation and caspase activation (Rintahaka et al. 2011). Actually, any non-specific binding that is able to induce innate immune systems can kill the cell. Some of the reason is concealed under non-specific or off-target effects of delivered siRNAs.

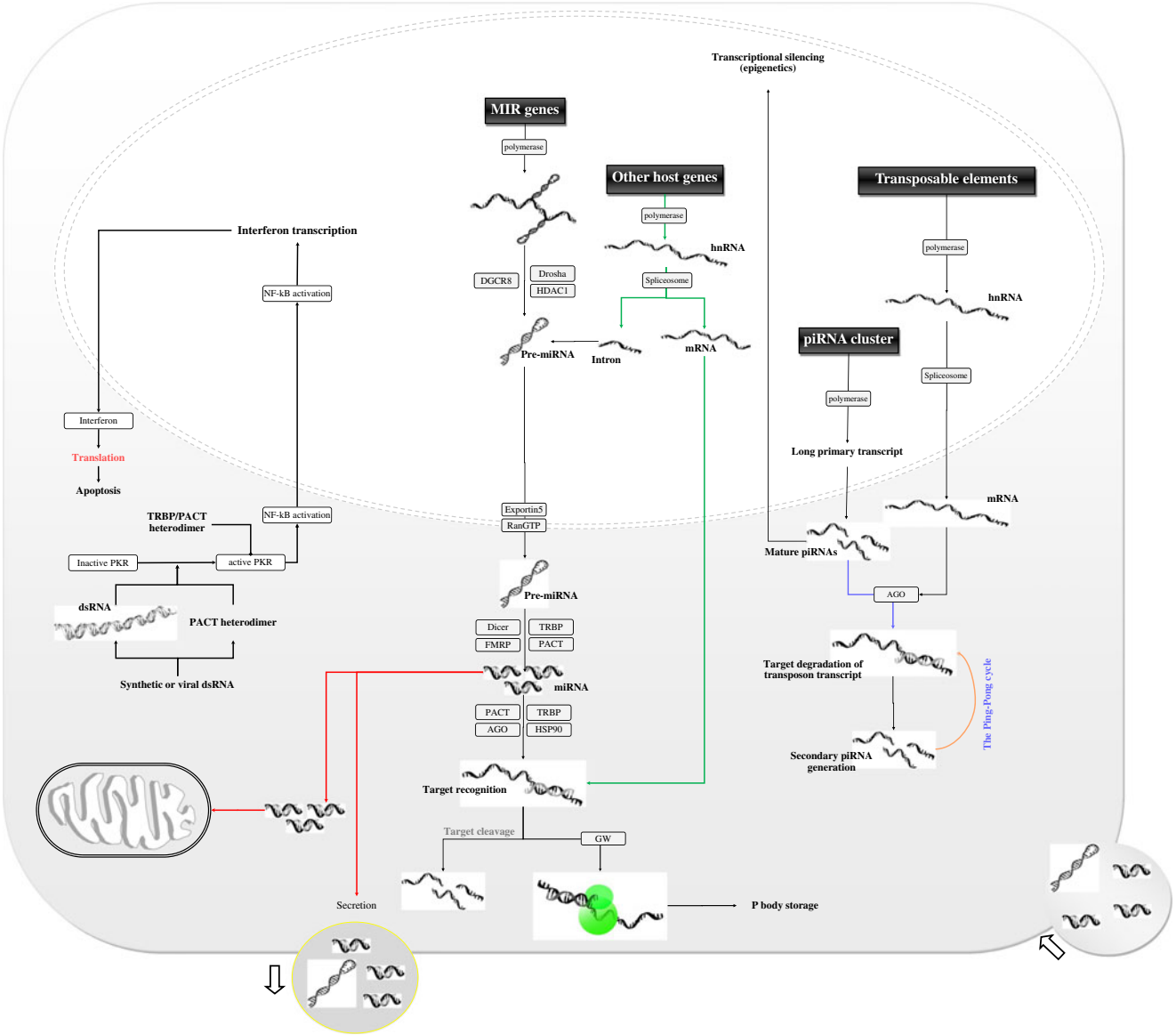

Fig. 5 Integrated small RNA-based silencing pathways in human (high resolution picture has been uploaded as Supplementary file in Microsoft Publisher format)

Innate immune system is always *on* even before infection. It is non-specific and is considered as the first line of immune defense layer. One aspect of this defense system is its sensitivity to viral proteins and nucleic acids distinguishing them from host components. Pattern recognition receptors are important proteins that can distinguish host and foreign components. They are located in the cytoplasm and in the cell membrane. In this system, there is a report on "dsRNA induced general silencing pathway" which senses viral or similar dsRNAs and induces cell death (Fig. 4). Retinoic-acid-inducible gene-1 (RIG-1) is one of those that recognize dsRNAs. After binding this substrate, it induces cytokine secretion causing primary signaling molecules in inflammation. Likewise, Toll-like receptors (TLRs) can identify ssDNA, dsRNA, and glycoproteins. Both RIG-1 and TLRs result in cytokine secretion but via different pathways including interferon-alpha, interferon-beta, TNF-alpha, IL-6, IL-12, and IFN-gamma. Locally, cytokines can initiate pathways by diffusing and systemically by entering to the blood circulation system. Systemic signaling will be observed phenotypically as pain, fever, loss of appetite, etc. Cytokines released by infected cells will be sensed by dendritic cells and macrophages and induce more cytokine release and secretion.

After detecting special dsRNAs (viral blunt-ended dsRNA with or without 5′ triphosphate, single-stranded RNA marked by a 5′-ppp and by polyuridine sequences), RIG-1 is activated and triggers interferon expression, and the general result was protein synthesis block and cell death. The RIG-1 conformation is changed after binding to dsRNA and ATP making it able to

induce a cascade of innate immune response including defense and inflammation (Kowalinski et al. 2011; Jiang et al. 2011).

PKR and interferon are also a component of viral dsRNA-induced pathway (Espada-Murao and Morita 2011; Boonyaratanakornkit et al. 2011). Early after viral infection, released dsRNA activates PRK (PKP-p) which phosphorylates translation transcription factor-2 (decreasing mRNA expression) and induces the expression of IFN. Many proteins including TRBP inhibits PKR, but PACT activates PKR, too (Daher et al. 2009). Some tricky viruses like HIV type 1 produce PKR inhibitors escaping RNAi monitoring system (Daher et al. 2009). Researches show that dsRNA can activate p38 and ERK1/2 which control IFN-beta autocrine secretion and cell death effect. Viral dsRNAs can do the same (Yu et al. 2011).

Conclusions

An updated RNAi-related pathway in the form of a super-pathway to show missing points and also indicating the plasticity of the pathway in human is provided in Fig. 5. Dysregulation of these pathways can be expected to be observed as complex diseases such as cancer and neuro-degeneration. Currently, more than 2,200 unique miRNAs reports are registered for human. The link between RNA silencing, RNA activation, and uncovering their connection and migration into with organelles and near/far cells are exciting research areas in the future. Surprisingly, the secretion of miRNA into milk and the reports of the existence of cross-kingdom miRNAs in human serum samples can make this super-pathway more complex.

Beyond their regulatory role within cytoplasm and nucleus, they can transmit into mitochondria (Ro et al. 2013; Lung et al. 2006). Surprisingly, they are released from the donor cells toward specific cells. This is a new horizon in RNA biology in which small RNAs are nominated as new multi-way cell–cell signaling strategies totally different from hormone–receptor or cytokines signaling. Scientists are trying to find out more about this mystified process to shed light on new drugs and also drug delivery systems. This is why there are huge investments in RNAi-based drug developments by giant companies. Recent reports are trying to find a relation among RNAi pathway components as an evidence of a specific pathobiology (biomarkers) such as various cancers in human (Papachristou et al. 2012). They can be reliable diagnostic, prognostic, and therapeutic biomarkers.

However, there are many unanswered questions regarding small RNAs biology, but there are promising standpoints about a new generation of drugs, strong and specific delivery methods, and ideal biomarkers. Growing reports on exon-skipping strategies to bypass genetic mutations in dystrophin mutations and restore its natural function in vitro (Fletcher et al. 2012; Adkin et al. 2012) indicate that many drugs and drug delivery strategies are developing based on small non-coding RNAs and also mimicking their mechanism of action. Therefore, every new finding in this area can guide us toward therapeutics which tries to mimic the small RNA inter- and intracellular spreading mechanisms as oligonucleotide drugs (Mitrpant et al. 2013).

References

Adkin CF et al (2012) Multiple exon skipping strategies to by-pass dystrophin mutations. Neuromuscul Disord 22(4):297–305

Ahlenstiel CL et al (2012) Direct evidence of nuclear Argonaute distribution during transcriptional silencing links the actin cytoskeleton to nuclear RNAi machinery in human cells. Nucleic Acids Res 40(4):1579–1595

Ando Y et al (2011) Two-step cleavage of hairpin RNA with 5′ overhangs by human DICER. BMC Mol Biol 12:6

Aravin AA et al (2001) Double-stranded RNA-mediated silencing of genomic tandem repeats and transposable elements in the D. melanogaster germline. Curr Biol 11(13):1017–1027

Aravin AA et al (2003) The small RNA profile during *Drosophila melanogaster* development. Dev Cell 5(2):337–350

Aravin A et al (2006) A novel class of small RNAs bind to MILI protein in mouse testes. Nature 442(7099):203–207

Aravin AA, Hannon GJ, Brennecke J (2007a) The Piwi-piRNA pathway provides an adaptive defense in the transposon arms race. Science 318(5851):761–764

Aravin AA et al (2007b) Developmentally regulated piRNA clusters implicate MILI in transposon control. Science 316(5825):744–747

Arroyo JD et al (2011) Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci U S A 108(12):5003–5008

Azimzadeh Jamalkandi S, Masoudi-Nejad A (2009) Reconstruction of *Arabidopsis thaliana* fully integrated small RNA pathway. Funct Integr Genomics 9(4):419–432

Azimzadeh Jamalkandi S, Masoudi-Nejad A (2011) RNAi pathway integration in *Caenorhabditis elegans* development. Funct Integr Genomics 11(3):389–405

Azuma-Mukai A et al (2008) Characterization of endogenous human Argonautes and their miRNA partners in RNA silencing. Proc Natl Acad Sci U S A 105(23):7964–7969

Bandres E et al (2009) MicroRNA-451 regulates macrophage migration inhibitory factor production and proliferation of gastrointestinal cancer cells. Clin Cancer Res 15(7):2281–2290

Barr I et al (2011) DiGeorge critical region 8 (DGCR8) is a double-cysteine-ligated heme protein. J Biol Chem 286(19):16716–16725

Barr I et al (2012) Ferric, not ferrous, heme activates RNA-binding protein DGCR8 for primary microRNA processing. Proc Natl Acad Sci U S A 109(6):1919–1924

Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116(2):281–297

Benhamed M et al (2012) Senescence is an endogenous trigger for microRNA-directed transcriptional gene silencing in human cells. Nat Cell Biol 14(3):266–275

Bennasser Y et al (2011) Competition for XPO5 binding between Dicer mRNA, pre-miRNA and viral RNA regulates human Dicer levels. Nat Struct Mol Biol 18(3):323–327

Bogerd HP et al (2010) A mammalian herpesvirus uses noncanonical expression and processing mechanisms to generate viral MicroRNAs. Mol Cell 37(1):135–142

Bohnsack MT, Czaplinski K, Gorlich D (2004) Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA 10(2):185–191

Boonyaratanakornkit J et al (2011) The C proteins of human parainfluenza virus type 1 limit double-stranded RNA accumulation that would otherwise trigger activation of MDA5 and protein kinase R. J Virol 85(4):1495–1506
Brennecke J et al (2005) Principles of microRNA-target recognition. PLoS Biol 3(3):e85
Brodersen P, Voinnet O (2009) Revisiting the principles of microRNA target recognition and mode of action. Nat Rev Mol Cell Biol 10(2):141–148
Brownawell AM, Macara IG (2002) Exportin-5, a novel karyopherin, mediates nuclear export of double-stranded RNA binding proteins. J Cell Biol 156(1):53–64
Calado A et al (2002) Exportin-5-mediated nuclear export of eukaryotic elongation factor 1A and tRNA. EMBO J 21(22):6216–6224
Chakravarthy S et al (2010) Substrate-specific kinetics of Dicer-catalyzed RNA processing. J Mol Biol 404(3):392–402
Cheloufi S et al (2010) A dicer-independent miRNA biogenesis pathway that requires Ago catalysis. Nature 465(7298):584–589
Chen T, Brownawell AM, Macara IG (2004) Nucleocytoplasmic shuttling of JAZ, a new cargo protein for exportin-5. Mol Cell Biol 24(15):6608–6619
Chen X et al (2008) Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 18(10):997–1006
Chendrimada TP et al (2005) TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. Nature 436(7051): 740–744
Chi YH, Semmes OJ, Jeang KT (2011) A proteomic study of TAR-RNA binding protein (TRBP)-associated factors. Cell Biosci 1(1):9
Chu Y et al (2010) Involvement of argonaute proteins in gene silencing and activation by RNAs complementary to a non-coding transcript at the progesterone receptor promoter. Nucleic Acids Res 38(21): 7736–7748
Chu Y et al (2012) Transcriptional silencing by hairpin RNAs complementary to a gene promoter. Nucleic Acid Ther 22(3):147–151
Chueh AC et al (2009) LINE retrotransposon RNA is an essential structural and functional epigenetic component of a core neocentromeric chromatin. PLoS Genet 5(1):e1000354
Cocucci E, Racchetti G, Meldolesi J (2009) Shedding microvesicles: artefacts no more. Trends Cell Biol 19(2):43–51
Corcoran DL et al (2009) Features of mammalian microRNA promoters emerge from polymerase II chromatin immunoprecipitation data. PLoS One 4(4):e5279
Courts C, Madea B (2011) Specific micro-RNA signatures for the detection of saliva and blood in forensic body-fluid identification. J Forensic Sci 56(6):1464–1470
Curtis HJ, Sibley CR, Wood MJ (2012) Mirtrons, an emerging class of atypical miRNA. Wiley Interdiscip Rev RNA 3:617–632
Daher A et al (2009) TRBP control of PACT-induced phosphorylation of protein kinase R is reversed by stress. Mol Cell Biol 29(1):254–265
Dallas A et al (2012) Right- and left-loop short shRNAs have distinct and unusual mechanisms of gene silencing. Nucleic Acids Res Advance access:1–17
Daniels SM et al (2009) Characterization of the TRBP domain required for dicer interaction and function in RNA interference. BMC Mol Biol 10:38
Detzer A et al (2011) Cell stress is related to re-localization of Argonaute 2 and to decreased RNA interference in human cells. Nucleic Acids Res 39(7):2727–2741
Doench JG, Sharp PA (2004) Specificity of microRNA target selection in translational repression. Genes Dev 18(5):504–511
El-Hefnawy T et al (2004) Characterization of amplifiable, circulating RNA in plasma and its potential as a tool for cancer diagnostics. Clin Chem 50(3):564–573
Elkayam E et al (2012) The structure of human argonaute-2 in complex with miR-20a. Cell 150(1):100–110
Espada-Murao LA, Morita K (2011) Delayed cytosolic exposure of Japanese encephalitis virus double-stranded RNA impedes interferon activation and enhances viral dissemination in porcine cells. J Virol 85(13):6736–6749
Esposito T et al (2011) piR_015520 belongs to Piwi-associated RNAs regulates expression of the human melatonin receptor 1A gene. PLoS One 6(7):e22727
Faller M et al (2010) DGCR8 recognizes primary transcripts of microRNAs through highly cooperative binding and formation of higher-order structures. RNA 16(8):1570–1583
Feng Y et al (2011) Drosha processing controls the specificity and efficiency of global microRNA expression. Biochim Biophys Acta 1809(11–12):700–707
Fleischhacker M, Schmidt B (2007) Circulating nucleic acids (CNAs) and cancer—a survey. Biochim Biophys Acta 1775(1):181–232
Fletcher S et al (2012) Targeted exon skipping to address "leaky" mutations in the dystrophin gene. Mol Ther Nucleic Acids 1:e48
Franks TM, Lykke-Andersen J (2008) The control of mRNA decapping and P-body formation. Mol Cell 32(5):605–615
Fukagawa T et al (2004) Dicer is essential for formation of the heterochromatin structure in vertebrate cells. Nat Cell Biol 6(8): 784–791
Galitskii VA (2008) Hypothesis of the initiation of DNA methylation de novo and allelic exclusion by small RNA. Tsitologiia 50(4):277–286
Gallo A et al (2012) The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. PLoS One 7(3):e30679
Gao Q, Frohman MA (2012) Roles for the lipid-signaling enzyme MitoPLD in mitochondrial dynamics, piRNA biogenesis, and spermatogenesis. BMB Rep 45(1):7–13
Gidlof O et al (2011) Cardiospecific microRNA plasma levels correlate with troponin and cardiac function in patients with ST elevation myocardial infarction, are selectively dependent on renal elimination, and can be detected in urine samples. Cardiology 118(4):217–226
Gokhale S et al (2012) A simple method for incorporating dynamic effects of intronic miRNA mediated regulation. Mol Biosyst 8(8):2145–2152
Gomez-Cabello D et al (2010) Regulation of the microRNA processor DGCR8 by the tumor suppressor ING1. Cancer Res 70(5):1866–1874
Gong M et al (2012) Caspases cleave and inhibit the microRNA processing protein DiGeorge Critical Region 8. Protein Sci 21(6): 797–808
Gregory RI et al (2004) The Microprocessor complex mediates the genesis of microRNAs. Nature 432(7014):235–240
Gu A et al (2010) Genetic variants in Piwi-interacting RNA pathway genes confer susceptibility to spermatogenic failure in a Chinese population. Hum Reprod 25(12):2955–2961
Gupta A et al (2006) Anti-apoptotic function of a microRNA encoded by the HSV-1 latency-associated transcript. Nature 442(7098):82–85
Gwizdek C et al (2004) Minihelix-containing RNAs mediate exportin-5-dependent nuclear export of the double-stranded RNA-binding protein ILF3. J Biol Chem 279(2):884–891
Han J et al (2004) The Drosha–DGCR8 complex in primary microRNA processing. Genes Dev 18(24):3016–3027
Han J, Kim D, Morris KV (2007) Promoter-associated RNA is required for RNA-directed transcriptional gene silencing in human cells. Proc Natl Acad Sci U S A 104(30):12422–12427
Han J et al (2009) Posttranscriptional crossregulation between Drosha and DGCR8. Cell 136(1):75–84
Hanke M et al (2010) A robust methodology to study urine microRNA as tumor marker: microRNA-126 and microRNA-182 are related to urinary bladder cancer. Urol Oncol 28(6):655–661
Hausser J et al (2009) Relative contribution of sequence and structure features to the mRNA binding of Argonaute/EIF2C-miRNA complexes and the degradation of miRNA targets. Genome Res 19(11):2009–2020

Havens MA et al (2012) Biogenesis of mammalian microRNAs by a non-canonical processing pathway. Nucleic Acids Res 40(10):4626–4640
Hawkins PG et al (2009) Promoter targeted small RNAs induce long-term transcriptional gene silencing in human cells. Nucleic Acids Res 37(9):2984–2995
Huang V et al (2010) RNAa is conserved in mammalian cells. PLoS One 5(1):e8848
Huang H et al (2011) piRNA-associated germline nuage formation and spermatogenesis require MitoPLD profusogenic mitochondrial-surface lipid signaling. Dev Cell 20(3):376–387
Huang V et al (2012) Upregulation of cyclin B1 by miRNA and its implications in cancer. Nucleic Acids Res 40(4):1695–1707
Iguchi H, Kosaka N, Ochiya T (2010) Secretory microRNAs as a versatile communication tool. Commun Integr Biol 3(5):478–481
Ikeda K et al (2006) Detection of the argonaute protein Ago2 and microRNAs in the RNA induced silencing complex (RISC) using a monoclonal antibody. J Immunol Methods 317(1–2):38–44
Iwasaki S, Tomari Y (2009) Argonaute-mediated translational repression (and activation). Fly (Austin) 3(3):204–206
Jagannath A, Wood MJ (2009) Localization of double-stranded small interfering RNA to cytoplasmic processing bodies is Ago2 dependent and results in up-regulation of GW182 and Argonaute-2. Mol Biol Cell 20(1):521–529
Janowski BA et al (2006) Involvement of AGO1 and AGO2 in mammalian transcriptional silencing. Nat Struct Mol Biol 13(9): 787–792
Janowski BA et al (2007) Activating gene expression in mammalian cells with promoter-targeted duplex RNAs. Nat Chem Biol 3(3):166–173
Jiang F et al (2011) Structural basis of RNA recognition and activation by innate immune receptor RIG-I. Nature 479(7373):423–427
Johnston M et al (2010) HSP90 protein stabilizes unloaded argonaute complexes and microscopic P-bodies in human cells. Mol Biol Cell 21(9):1462–1469
Kedersha NL et al (1999) RNA-binding proteins TIA-1 and TIAR link the phosphorylation of eIF-2 alpha to the assembly of mammalian stress granules. J Cell Biol 147(7):1431–1442
Kim DH et al (2006) Argonaute-1 directs siRNA-mediated transcriptional gene silencing in human cells. Nat Struct Mol Biol 13(9):793–797
Kim DH et al (2008) MicroRNA-directed transcriptional gene silencing in mammalian cells. Proc Natl Acad Sci U S A 105(42):16230–16235
Kok KH et al (2007) Human TRBP and PACT directly interact with each other and associate with dicer to facilitate the production of small interfering RNA. J Biol Chem 282(24):17649–17657
Koscianska E, Starega-Roslan J, Krzyzosiak WJ (2011) The role of Dicer protein partners in the processing of microRNA precursors. PLoS One 6(12):e28548
Kowalinski E et al (2011) Structural basis for the activation of innate immune pattern-recognition receptor RIG-I by viral RNA. Cell 147(2):423–435
Kozaki K, Inazawa J (2012) Tumor-suppressive microRNA silenced by tumor-specific DNA hypermethylation in cancer cells. Cancer Sci 103(5):837–845
Kuwabara T et al (2005) The NRSE smRNA specifies the fate of adult hippocampal neural stem cells. Nucleic Acids Symp Ser (Oxf) (49): 87–88
Kwak PB, Tomari Y (2012) The N domain of Argonaute drives duplex unwinding during RISC assembly. Nat Struct Mol Biol 19(2):145–151
Laraki G et al (2008) Interactions between the double-stranded RNA-binding proteins TRBP and PACT define the Medipal domain that mediates protein-protein interactions. RNA Biol 5(2):92–103
Lau PW et al (2009) Structure of the human Dicer-TRBP complex by electron microscopy. Structure 17(10):1326–1332
Lee RC, Feinbaum RL, Ambros V (1993) The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75(5):843–854
Lee YS et al (2009) A novel class of small RNAs: tRNA-derived RNA fragments (tRFs). Genes Dev 23(22):2639–2649
Lemaire PA et al (2008) Mechanism of PKR activation by dsRNA. J Mol Biol 381(2):351–360
Leung AK, Calabrese JM, Sharp PA (2006) Quantitative analysis of Argonaute protein reveals microRNA-dependent localization to stress granules. Proc Natl Acad Sci U S A 103(48):18125–18130
Li LC et al (2006) Small dsRNAs induce transcriptional activation in human cells. Proc Natl Acad Sci U S A 103(46):17337–17342
Li SC, Tang P, Lin WC (2007) Intronic microRNA: discovery and biological implications. DNA Cell Biol 26(4):195–207
Liao JY et al (2010) Deep sequencing of human nuclear and cytoplasmic small RNAs reveals an unexpectedly complex subcellular distribution of miRNAs and tRNA 3′ trailers. PLoS One 5(5): e10563
Lin YT, Sullivan CS (2011) Expanding the role of Drosha to the regulation of viral gene expression. Proc Natl Acad Sci U S A 108(27):11229–11234
Liu J et al (2004) Argonaute2 is the catalytic engine of mammalian RNAi. Science 305(5689):1437–1441
Lung B et al (2006) Identification of small non-coding RNAs from mitochondria and chloroplasts. Nucleic Acids Res 34(14):3842–3852
Ma N et al (2011) Coexpression of an intronic microRNA and its host gene reveals a potential role for miR-483-5p as an IGF2 partner. Mol Cell Endocrinol 333(1):96–101
Ma E et al (2012) Coordinated activities of human dicer domains in regulatory RNA processing. J Mol Biol 422:466–476
Maroney PA, Yu Y, Nilsen TW (2006) MicroRNAs, mRNAs, and translation. Cold Spring Harb Symp Quant Biol 71:531–535
Matranga C et al (2005) Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes. Cell 123(4):607–620
Meckes DG Jr et al (2010) Human tumor virus utilizes exosomes for intercellular communication. Proc Natl Acad Sci U S A 107(47): 20370–20375
Meister G et al (2004) Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. Mol Cell 15(2):185–197
Michael A et al (2010) Exosomes from human saliva as a source of microRNA biomarkers. Oral Dis 16(1):34–38
Minor J et al (2012) Methylation of microRNA-9 is a specific and sensitive biomarker for oral and oropharyngeal squamous cell carcinomas. Oral Oncol 48(1):73–78
Mitchell JP et al (2008) Increased exosome production from tumour cell cultures using the Integra CELLine Culture System. J Immunol Methods 335(1–2):98–105
Mitrpant C et al (2013) Improved antisense oligonucleotide design to suppress aberrant SMN2 gene transcript processing: towards a treatment for spinal muscular atrophy. PLoS One 8(4):e62114
Mohammad F et al (2012) Long noncoding RNA-mediated maintenance of DNA methylation and transcriptional gene silencing. Development 139(15):2792–2803
Monteys AM et al (2010) Structure and activity of putative intronic miRNA promoters. RNA 16(3):495–505
Morris KV et al (2004) Small interfering RNA-induced transcriptional gene silencing in human cells. Science 305(5688):1289–1292
Mueller C et al (2012) Sustained miRNA-mediated knockdown of mutant AAT with simultaneous augmentation of wild-type AAT has minimal effect on global liver miRNA profiles. Mol Ther 20(3): 590–600
Noland CL, Ma E, Doudna JA (2011) siRNA repositioning for guide strand selection by human Dicer complexes. Mol Cell 43(1):110–121
Ohrt T et al (2006) In situ fluorescence analysis demonstrates active siRNA exclusion from the nucleus by Exportin 5. Nucleic Acids Res 34(5):1369–1380
Okamura K, Lai EC (2008) Endogenous small interfering RNAs in animals. Nat Rev Mol Cell Biol 9(9):673–678

Omel'ianchuk NA, Ponomarenko PM, Ponomarenko MP (2011) The nucleotide sequence features of the mature microRNA seem to be responsible for the affinity to human Ago2 AND Ago3 proteins. Mol Biol (Mosk) 45(2):366–375
Ono M et al (2011) Identification of human miRNA precursors that resemble box C/D snoRNAs. Nucleic Acids Res 39(9): 3879–3891
Pan W et al (2010) MicroRNA-21 and microRNA-148a contribute to DNA hypomethylation in lupus CD4+ T cells by directly and indirectly targeting DNA methyltransferase 1. J Immunol 184(12): 6773–6781
Papachristou DJ et al (2012) Immunohistochemical analysis of the endoribonucleases Drosha, Dicer and Ago2 in smooth muscle tumours of soft tissues. Histopathology 60(6B):E28–E36
Pare JM et al (2009) Hsp90 regulates the function of argonaute 2 and its recruitment to stress granules and P-bodies. Mol Biol Cell 20(14): 3273–3284
Park NJ et al (2009) Salivary microRNA: discovery, characterization, and clinical utility for oral cancer detection. Clin Cancer Res 15(17): 5473–5477
Park JE et al (2011) Dicer recognizes the 5′ end of RNA for efficient and accurate processing. Nature 475(7355):201–205
Parsi S et al (2012) Experimental verification of a predicted intronic microRNA in human NGFR gene with a potential pro-apoptotic function. PLoS One 7(4):e35561
Patel RS et al (2011) High resolution of microRNA signatures in human whole saliva. Arch Oral Biol 56(12):1506–1513
Pauler FM, Koerner MV, Barlow DP (2007) Silencing by imprinted noncoding RNAs: is transcription the answer? Trends Genet 23(6): 284–292
Perron MP, Provost P (2009) Protein components of the microRNA pathway and human diseases. Methods Mol Biol 487:369–385
Place RF et al (2008) MicroRNA-373 induces expression of genes with complementary promoter sequences. Proc Natl Acad Sci U S A 105(5):1608–1613
Place RF et al (2010) Defining features and exploring chemical modifications to manipulate RNAa activity. Curr Pharm Biotechnol 11(5):518–526
Portnoy V et al (2011) Small RNA and transcriptional upregulation. Wiley Interdiscip Rev RNA 2(5):748–760
Regazzi R, Widmann C (2012) Genetics and molecular biology: miRNAs take the HDL ride. Curr Opin Lipidol 23(2):165–166
Rintahaka J et al (2011) Recognition of cytoplasmic RNA results in cathepsin-dependent inflammasome activation and apoptosis in human macrophages. J Immunol 186(5):3085–3092
Ro S et al (2013) The mitochondrial genome encodes abundant small noncoding RNAs. Cell Res 23(6):759–774
Rosenfeld JA et al (2009) Determination of enriched histone modifications in non-genic portions of the human genome. BMC Genomics 10:143
Ruby JG, Jan CH, Bartel DP (2007) Intronic microRNA precursors that bypass Drosha processing. Nature 448(7149):83–86
Rudel S, Meister G (2008) Phosphorylation of Argonaute proteins: regulating gene regulators. Biochem J 413(3):e7–e9
Rudel S et al (2008) A multifunctional human Argonaute2-specific monoclonal antibody. RNA 14(6):1244–1253
Rudel S et al (2011) Phosphorylation of human Argonaute proteins affects small RNA binding. Nucleic Acids Res 39(6):2330–2343
Sakurai K et al (2011) A role for human Dicer in pre-RISC loading of siRNAs. Nucleic Acids Res 39(4):1510–1525
Sarkar SN, Sen GC (2004) Novel functions of proteins encoded by viral stress-inducible genes. Pharmacol Ther 103(3):245–259
Schirle NT, MacRae IJ (2012) The crystal structure of human Argonaute2. Science 336(6084):1037–1040
Schopman NC et al (2010) A miRNA-tRNA mix-up: tRNA origin of proposed miRNA. RNA Biol 7(5):573–576
Scian MJ et al (2011) MicroRNA profiles in allograft tissues and paired urines associate with chronic allograft dysfunction with IF/TA. Am J Transplant 11(10):2110–2122
Scott MS, Ono M (2011) From snoRNA to miRNA: dual function regulatory non-coding RNAs. Biochimie 93(11):1987–1992
Sen GL, Blau HM (2005) Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies. Nat Cell Biol 7(6):633–636
Shenoy A, Blelloch R (2009) Genomic analysis suggests that mRNA destabilization by the microprocessor is specialized for the auto-regulation of Dgcr8. PLoS One 4(9):e6971
Sigurdsson MI et al (2012) The distribution of a germline methylation marker suggests a regional mechanism of LINE-1 silencing by the piRNA-PIWI system. BMC Genet 13:31
Singh M et al (2011) Stress-induced phosphorylation of PACT reduces its interaction with TRBP and leads to PKR activation. Biochemistry 50(21):4550–4560
Skalsky RL et al (2012) The viral and cellular microRNA targetome in lymphoblastoid cell lines. PLoS Pathog 8(1):e1002484
Stefani G, Slack FJ (2012) A 'pivotal' new rule for microRNA-mRNA interactions. Nat Struct Mol Biol 19(3):265–266
Stern-Ginossar N et al (2007) Host immune system gene targeting by a viral miRNA. Science 317(5836):376–381
Su H et al (2009) Essential and overlapping functions for mammalian Argonautes in microRNA silencing. Genes Dev 23(3):304–317
Suzuki Y et al (2009) The Hsp90 inhibitor geldanamycin abrogates colocalization of eIF4E and eIF4E-transporter into stress granules and association of eIF4E with eIF4G. J Biol Chem 284(51):35597–35604
Svoboda P et al (2004) RNAi and expression of retrotransposons MuERV-L and IAP in preimplantation mouse embryos. Dev Biol 269(1):276–285
Tahbaz N et al (2004) Characterization of the interactions between mammalian PAZ PIWI domain proteins and Dicer. EMBO Rep 5(2):189–194
Takimoto K, Wakiyama M, Yokoyama S (2009) Mammalian GW182 contains multiple Argonaute-binding sites and functions in microRNA-mediated translational repression. RNA 15(6):1078–1089
Tang R et al (2012) Mouse miRNA-709 directly regulates miRNA-15a/16-1 biogenesis at the posttranscriptional level in the nucleus: evidence for a microRNA hierarchy system. Cell Res 22(3):504–515
Ting AH et al (2008) A requirement for DICER to maintain full promoter CpG island hypermethylation in human cancer cells. Cancer Res 68(8):2570–2575
Triboulet R et al (2009) Post-transcriptional control of DGCR8 expression by the Microprocessor. RNA 15(6):1005–1011
Tsuchiya S et al (2009) MicroRNA-338-3p and microRNA-451 contribute to the formation of basolateral polarity in epithelial cells. Nucleic Acids Res 37(11):3821–3827
Turner AM et al (2012) Characterization of an HIV-targeted transcriptional gene-silencing RNA in primary cells. Hum Gene Ther 23(5):473–483
Valadi H et al (2007) Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 9(6):654–659
Valdmanis PN et al (2012) Expression determinants of mammalian argonaute proteins in mediating gene silencing. Nucleic Acids Res 40(8):3704–3713
Vickers KC et al (2011) MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat Cell Biol 13(4):423–433
von Brandenstein M et al (2012) MicroRNA 15a, inversely correlated to PKCalpha, is a potential marker to differentiate between benign and malignant renal tumors in biopsy and urine samples. Am J Pathol 180(5):1787–1797

Wada T, Kikuchi J, Furukawa Y (2012) Histone deacetylase 1 enhances microRNA processing via deacetylation of DGCR8. EMBO Rep 13(2):142–149

Wang Z et al (2011) DNA hypermethylation of microRNA-34b/c has prognostic value for stage non-small cell lung cancer. Cancer Biol Ther 11(5):490–496

Wang LL et al (2012a) The potential role of microRNA-146 in Alzheimer's disease: biomarker or therapeutic target? Med Hypotheses 78(3):398–401

Wang X et al (2012b) Induction of NANOG expression by targeting promoter sequence with small activating RNA antagonizes retinoic acid-induced differentiation. Biochem J 443(3):821–828

Watanabe T et al (2006) Identification and characterization of two novel classes of small RNAs in the mouse germline: retrotransposon-derived siRNAs in oocytes and germline small RNAs in testes. Genes Dev 20(13):1732–1743

Wee EJ et al (2012) Mapping the regulatory sequences controlling 93 breast cancer-associated miRNA genes leads to the identification of two functional promoters of the Hsa-mir-200b cluster, methylation of which is associated with metastasis or hormone receptor status in advanced breast cancer. Oncogene 31:4182–4195

Weinberg MS et al (2006) The antisense strand of small interfering RNAs directs histone methylation and transcriptional gene silencing in human cells. RNA 12(2):256–262

Wightman B, Ha I, Ruvkun G (1993) Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. Cell 75(5):855–862

Wong KY, Huang X, Chim C (2012) DNA methylation of microRNA genes in multiple myeloma. Carcinogenesis. doi:10.1093/carcin/bgs212

Wu YL et al (2011) A non-coding RNA of insect HzNV-1 virus establishes latent viral infection through microRNA. Sci Rep 1:60

Yan L et al (2011) Intronic microRNA suppresses endothelial nitric oxide synthase expression and endothelial cell proliferation via inhibition of STAT3 signaling. Mol Cell Biochem 357(1–2):9–19

Yang JS et al (2010) Conserved vertebrate mir-451 provides a platform for Dicer-independent, Ago2-mediated microRNA biogenesis. Proc Natl Acad Sci U S A 107(34):15163–15168

Yang C et al (2012) Identification of seven serum microRNAs from a genome-wide serum microRNA expression profile as potential noninvasive biomarkers for malignant astrocytomas. Int J Cancer

Yi R et al (2003) Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev 17(24):3011–3016

Yi R et al (2005) Overexpression of exportin 5 enhances RNA interference mediated by short hairpin RNAs and microRNAs. RNA 11(2):220–226

Younger ST, Corey DR (2009) The puzzle of RNAs that target gene promoters. Chembiochem 10(7):1135–1139

Younger ST, Corey DR (2011) Transcriptional gene silencing in mammalian cells by miRNA mimics that target gene promoters. Nucleic Acids Res 39(13):5682–5691

Younger ST, Pertsemlidis A, Corey DR (2009) Predicting potential miRNA target sites within gene promoters. Bioorg Med Chem Lett 19(14):3791–3794

Yu N et al (2011) Double-stranded RNA induces melanocyte death via activation of Toll-like receptor 3. Exp Dermatol 20(2):134–139

Yu F et al (2012) MicroRNA 34c gene down-regulation via DNA methylation promotes self-renewal and epithelial-mesenchymal transition in breast tumor-initiating cells. J Biol Chem 287(1):465–473

Zahm AM et al (2012) Circulating MicroRNA is a biomarker of biliary Atresia. J Pediatr Gastroenterol Nutr 55:366–369

Zampetaki A et al (2012) Profiling of circulating microRNAs: from single biomarkers to re-wired networks. Cardiovasc Res 93(4):555–562

Zen K, Zhang CY (2012) Circulating microRNAs: a novel class of biomarkers to diagnose and monitor human cancers. Med Res Rev 32(2):326–348

Zeng Y, Cullen BR (2004) Structural requirements for pre-microRNA binding and nuclear export by Exportin 5. Nucleic Acids Res 32(16):4776–4785

Zeng Y et al (2008) Phosphorylation of Argonaute 2 at serine-387 facilitates its localization to processing bodies. Biochem J 413(3):429–436

Zhang X, Zeng Y (2010) The terminal loop region controls microRNA processing by Drosha and Dicer. Nucleic Acids Res 38(21):7689–7697

Zhang X et al (2009) Alterations in miRNA processing and expression in pleomorphic adenomas of the salivary gland. Int J Cancer 124(12):2855–2863

Zhang Y et al (2010) Secreted monocytic miR-150 enhances targeted endothelial cell migration. Mol Cell 39(1):133–144

Zhao Z et al (2012) Circulating microRNA miR-323-3p as a biomarker of ectopic pregnancy. Clin Chem 58(5):896–905

Zhou H, Huang C, Xia XG (2008) A tightly regulated Pol III promoter for synthesis of miRNA genes in tandem. Biochim Biophys Acta 1779(11):773–779

Zhou X et al (2012a) MicroRNA-9 as potential biomarker for breast cancer local recurrence and tumor estrogen receptor status. PLoS One 7(6):e39011

Zhou J et al (2012b) Transcriptional gene silencing of HPV16 E6/E7 induces growth inhibition via apoptosis in vitro and in vivo. Gynecol Oncol 124(2):296–302