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MicroRNA Implication in Cancer

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Abstract

MicroRNAs (miRNA) are a new class of posttranscriptional regulators. These small non-coding RNAs regulate the expression of target mRNA transcripts and are linked to several human disease such as Alzheimer, cancer or heart disease. But it has been the cancer disease which has experimented the major number of studies of miRNA linked to the disease progression. In the last years it has been reported the deregulation pattern of the miRNAs in malignant cells which have disrupted the control of the proliferation, differentiation or apoptosis. The evidence of the presence of specific miRNA deregulated in concrete cancer types has become the miRNAs like possible biomarkers and therapeutic targets. The specific miRNA patterns deregulated in concrete cancer cell types open new opportunities to the diagnosis and therapy.

Keywords: microRNA, cancer, metastasis, post-transcription, biomarkers

Introduction

MicroRNAs (miRNA) were first discovered in 1993 by Victor Ambros and collaborators during a development study in the nematode *C. elegans* (Lee *et al.*, 1993). Although in the beginning this feature was considered a nematode idiosyncrasy, posterior studies demonstrated that it was well conserved in the species beyond nematodes (Pasquinelli *et al.*, 2000). They saw the presence of non-coding small RNAs that despite they do not translate in proteins they play key role in the final protein expression (Reinhart *et al.*, 2000). The microRNAs (miRNA) are short ~22 nucleotide RNA sequences that bind to complementary sequences in the 3' UTR of multiple target mRNAs, usually resulting in their silencing (Bartel, 2004). In the last decade the study of the miRNA has experimented an exponential development and more than 700 has been discovered and more than 700 are predicted to be studied (Bentwich *et al.*, 2005). But the regulatory systems of the miRNAs are more complex that it looks like. Each miRNA can repress hundreds of target mRNAs and it is not clear the minimum sequence complementary required for the silencing (Brennecke *et al.*, 2003; Lim *et al.*, 2005). The role of the miRNAs is not reduced to an unique cell process. The presence of the miRNAs has been described in very heterogenic cell processes such as differentiation, apoptosis, haemotopoiesis, fat metabolism, or limb morphogenesis (Brennecke *et al.*, 2003; Chen *et al.*, 2004; Cuellar and McManus, 2005; Harfe *et al.*, 2005; Poy *et al.*, 2004; Wilfred *et al.*, 2007). In the case of the diseases the deregulation pattern of miRNAs leads to a different protein expressions that finally results in different cellular capabilities. These capabilities can conferee to the cell a specific cell behavior than in the case of the cancer gives a malignant potential. For these reason the miRNA profiles have been well studied in the cancer biology. Until now, the oncogenes and tumor supressor genes have been considered the genes that code for a certain proteins, now the new trend is that these non-coding sequences could be considered like oncogenes or tumor supressor genes because their role in the final protein expression in the cancer evolve (He *et al.*, 2009). The miRNAs can actuate in both sense in cancer. They can repress proteins that are tumor promoters, in this case they would be considered tumor supressor genes like in the case of miR 146 (Hurst *et al.*, 2009), or could be oncogenes, downregulating tumor suppressor proteins like in the case of the miR-130a (Chen and Gorski, 2008). But the silencing machinery of the miRNAs is not composed only by the miRNA and the targeted mRNA. A cluster of proteins compiled in the RISC complex (RNA Induced Silencing Complex) work together in the catalytic process of the mRNA degradation step (Schwarz *et al.*, 2004).

MiRNA biogenesis and action

The miRNA are processed from much longer transcripts called pri-miRNA (Lee *et al.*, 2002). Although the first miRNA discovered were located in intergenic clusters within the genome (Lee *et al.*, 1993), nowadays more than 50 % of the characterized miRNAs are located in introns (Rodriguez *et al.*, 2004). Most of the pri-miRNA are transcribed by RNA polymerase II (Lee *et al.*, 2004) but also are some which are transcribed by polymerase III (Borchert *et al.*, 2006). The resulted sequence is processed by a complex called Microprocessor that contains RNA

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binding protein Pasha (Han *et al*., 2004) and the nuclear RNAse III type enzyme Drosha (Lee *et al*., 2003). In this process the pri-miRNA are converted in ~ 70 nucleotides sequence called pre-miRNA inside the nucleus and are exported to the cytoplasm via Exportin 5 (Yi *et al*., 2003). The pre-miRNA is cleavaged in the cytoplasm by another typeIII RNAse called Dicer resulting in ~ 20 nucleotide double strand RNA (Hutvagner *et al*., 2001). In this point still it is not well elucidated how from a double strand only one strand of RNA is charged in the RNA-Induced Silencing Complex (RISC). It is not clear which of the strands is loaded to the RISC, but it seems that the thermodynamycall stability play a key role in the selection, being the less stable strand the selected to be part of the complex (Krol, 2004). The RISC is a multiprotein complex but the core structure is composed by the Argonaute (Ago) protein family members called Ago 1-4. The Ago proteins contain three principle domains (Peters and Meister, 2007):

- Piwi-Argonaute-Zwille (PAZ) which mediates nucleic acid binding.
- Middle Domain (MID) is critical for the association between the RNA and the Ago.
- PIWI domain that contains the ribonuclease activity.

Between the 4 Ago proteins the Ago-2 is what has the catalytic function to degrade the target mRNA. But there is another interesting fact in the miRNA silencing process via RISC, which is the subcellular location of the RISC complex. The RISC complex actuate in determine subcellular structures called Processing bodies (P-bodies) (Liu *et al*., 2005). These are cytoplamic compartments consisting of many enzymes involved in mRNA turnover as well as the RISC actuation during mRNA silencing via miRNA.

MiRNA in the diseases

The role of the miRNAS in the regulation of the proteins in the healthy conditions is evident, thus the role of the miRNAs in the development is evident: miR-143 modulates the adypocytes differentiation (Esau *et al*., 2004), miR-124a and miR-9 contribute to neuronal stem cell differentiation and miR-181 is the responsible of the haematopoietic lineage differentiation (Chen and Gorski, 2004) and myoblast differentiation (Naguibneva *et al*., 2006). But the miRNAs are not only involved in the development, they also play a key role in the adult organ function, for instance miR-155 actuate in the lymphocyte function (Thai *et al*., 2007) and in the immune response (Rodriguez *et al*., 2007) and miR-375 regulates insulin secretion by the beta cells of the pancreas (Poy *et al*., 2004). Considering that the miRNAs are implicated in nearly all of the biological processes, they were considered for the scientist like a possible targets to study the different disease. The deletion of genetic fragments produce cancer disease like B cell chronic lymphocytic leukemia due to a deletion of a chromosomic fragments. The miR-15a and miR-16-1 are located in these fragments and these miRNA are involved in the maturation process of these cells, correlating the miRNA repercussion in the disease (Calin *et al*., 2002). Another disease related with loss of genetic material is the case of cerebral degeneration. It has been demonstrated that the downexpression of the Dicer protein in the Purkinje cells in the brain provokes less mature miRNA production and consequently the miRNA implicated in the brain cell maturation can not actuate (Schaefer *et al*., 2007). This loss of molecular activity end with an ataxia due to a maturation problem. Let-7 is another miRNA which loss is directly related with the lung cancer progression. It has been well documented that mentioned miRNA represses oncogene Ras that can provoke malignant tumors (Takamizawa *et al*., 2004). This concerning about the miRNA loosing related with disease progression. But even the gain of miRNA are related directly with disease progression; it is the case of some human cancer types that appears with an overexpression of miR-143 and miR-145 (Akao *et al*., 2006); or the presence of the miR-133 in diabetic patients is related with heart problems and it has been demonstrated that miR-133 repress HERG K+ channel expression contributing to QT prolongation that finally can produce heart problems (Xiao *et al*., 2007). In resume, studying the implication of the miRNAs in the different disease it is possible to elucidate that the regulation mechanism of the miRNA is a balance between a positive activation and negative repression. In some cases the gain of a miRNA provoke the repression of necessary protein for the normal function of the organ, and in other cases it is just the opposite effect, in which a downregulation of a repressor miRNA provoke an augmentation of a protein that conferee the malignancy to the cell progressing to the disease. These mechanisms can actuate in different disease but for the characteristics of gain and loss of protein expression and the tight relationship of the miRNAs with the differentiation processes it has been a good bank of studies and target picking area for cancer disease.

MiRNA in cancer disease

During the miRNA study it has been discovered many features that have direct relationship with the cancer biology: the correlation of some miRNAs like let-7 with the cell proliferation (Brennecke *et al*., 2003) or the fact that many miRNAs are located in the fragile sites in the genome in specific regions that suffer frequently amplifications or deletions (Calin *et al*., 2004). These facts push the scientist to study the miRNAs´s role in the cancer with many singular results that are cell and tissue specific. Since the first evidence of the involvement of a miRNA in the cancer pathogenesis was demonstrated in the case of the human chronic lymphocytic leukemia (CLL) in which miR-15 and miR-16 are absent or downregulated (Calin *et al*., 2002), many miRNA has been untapped like cancer origin. It is the case of the miR-17-92 cluster which affects

directly in *c-Myc* oncogen overexpression in some tumors provoking the cancer growing (He *et al*., 2005), and the same cluster can actuate like antiapoptotic element in other tissues (Matsubara *et al*., 2007). In both cases the final result is the tumor progression and the cluster could be considered like an oncogene, but the pathways in which the cluster is actuating are different. The other characteristic of the miRNAs is their capacity to actuate like tumor suppressors. It is the case of the let-7. Let-7 is the miRNA that silences the RAS protein. The RAS protein is a membrane-associated signaling protein that regulates the cell proliferation. The overexpression of this protein is correlated with tumor growing. The let-7 actuates suppressing the RAS expression, so the let-7 absence produces the RAS overexpression and cancer cell proliferation (Johnson *et al*., 2005). This is the case the miRNA is actuating like tumor suppressor element. But the general overview of a miRNA profiling study shows that the cancer cells have less miRNAs than normal tissues suggesting that the pivotal role of the miRNA is to drive the cell differentiation and prevent the cell division (Gaur *et al*., 2007; Lu *et al*., 2005). To demonstrate this feature Kumar *et al.* (2007) developed Dicer mutant mice. The loss of Dicer function resulted in the reduction of mature miRNAs and less amount of miRNAs; and these mice developed more tumors than the wild types (Kumar *et al*., 2007). These are the cases in which the miRNAs actuate directly in the biological pathways, but also there are cases in those the protein signaling is actuating in the expression of certain miRNAs. This is the case of p53 and miR-34. The p53 is considered "the guardian of the genome" because is the responsible of the regulation of the cell proliferation. In stress situations, p53 actuates and prevents the excessive cell proliferation. It has been demonstrated in various works that the p53 activation upregulates the miR34 and this element arise the cell cycle arrest or apoptosis (He *et al*., 2007; Chang *et al*., 2007). But this is a general overview of the miRNAs implication in the cancer biology. And as we know cancer is not an unique disease. There are more than 200 cancer types that can differ depending from which tissue they come from. Even each cancer type develops in different subtypes depending on the malignancy and from which the cell type are originated. When we are going to speak about cancer the general features are the same for all of them but the proteins, pathways and genes implicated in them are specific for each type. To do a review it is essential to take one type like a model and focus on the networks and implications of the different elements involved in it. One of the most studied cancer types for its incidence in the population is the breast cancer. It is the most common cancer types in women and the malignancy and drug resistance is so important that a lot of efforts have been put in the study of the breast cancer. Also the miRNAs have been studied in the breast cancer, and nowadays the panel of all of these elements and the networks with the proteins implicated in the disease are huge. Like we said previously, these results can not be transferred exactly to other cancer types but it can be used like models that can approximate to another cancer types and can help to understand and localize cancer biomarkers and therapeutic targets. For these reason we will review the miRNA implication in the breast cancer like cancer model.

Breast cancer a suitable model for miRNA implication study

The type of miRNAs studies in the breast cancers are broad, but it is interesting to start with the research focused on the miRNA location in the genome. Iorio et al. and Zhang and colleagues demonstrated that the majority of miRNAs deregulated are located in genomic fragile sites. These sites suffers a lot of abnormalities such as deletions or excessive gene copies. For this reason the genomic fragile sites are correlated with the cancer, and this discovery began to demonstrate the key role of the miRNAs in the cancer biology (Calin *et al*., 2004; Iorio *et al*., 2005; Zhang *et al*., 2006). This study open the vision of the possible implication of the miRNAs in the cancer. The specific location in these fragile sites correlates with the upregulations and downregulation of these elements that finally result in the protein imbalance that conferee the proliferative and malignant behavior to the tumor cells. And it is the proliferation capacity of these cells and the possible implication of the miRNAs the other fact well studied in this field. The cell cycle alterations in the breast cancers concern mainly to: loss of retinoblastoma (Rb) function, reduced cyclin-dependentkinase (CDK) inhibitor p27 (Kip1) and p57(Kip2) abundances, as well as increased abundance of D and E type cyclins. One by one all the events have been correlated whit one or more miRNAs. The retinoblastoma (Rb) is the protein which regulates the cell cycle. The alteration or mutation of this protein provokes an inhibition of cell cycle regulation and the cells proliferate out of control. The cluster of miRNAs called miR-17/20 regulates the Rb final expression (Hossain *et al*., 2006). An alteration of miR 17/20 cluster results in altered Rb protein expression and finally the cell cycle is deregulated. The other event is the alteration of CDK inhibitors. MiR 221/222 regulate the cell cycle targeting the CDK inhibitors. The cell cycle in part is regulated by cyclines and inhibitors, and the correct balance of both results in the correct cell progression. If a CDK inhibitor is downregulated the CDK will actuate and the cell will go ahead in the proliferation. In some breast tumors the miR 221/222 target the CDK inhibitors p27 and p57 and facilitate the G_1/S phase transition allowing the tumor growth. (Kim *et al*., 2009; Miller *et al*., 2008). And finally the cyclins regulation. The miR-34 family composed by miR 34a/b/c actuate via p53 tumor suppressor. In stress conditions p53 is activated and suppress the cell proliferation. It has been demonstrated that p53 activated binds to miR-34 family promoters and induce the expression. The miR-34 targets

the cyclins D1 and E2 and provoke the cell cycle arrest. In proliferative breast tumor cells the p53 is altered and the miR-34 a/b/c are downregulated (He *et al.*, 2007; Tarasov *et al.*, 2007; Sun *et al.*, 2008).

The other essential event in the tumor malignancy progression is the capacity of the tumors to metastasize. The metastasis is the capacity of the tumor to invade adjacent tissues and growth like secondary tumor. But this is a complex process which requires a diversity of genetic changes. Event like migration and dedifferentiation are linked to this process and different miRNAs are involved in these properties. The CD44 is a protein which blocks the transformation of the cells to a metastatic phenotype. The miR-373 and miR 520c suppress the CD44 expression and permit to the cell to progress to a metastatic behavior (Huang *et al.*, 2008; Lopez *et al.*, 2008). Hoxd10 is another protein that blocks the migration capacity of the cells and in some breast tumors this protein is suppressed by miR-10b (Ma and Weinberg, 2007). In contrast the miR-335 target the transcription factor Sox4 which promote the cell migration, so the loss of miR-335 results in the augment of cell migration capacity (Tavazoie *et al.*, 2008). Another event that occurs during metastasis is the progressive lost of E-cadherins –proteins that maintain the cell unions- and the progression to a mesenchymal phenotype (epithelial mesenchymal transition or EMT). The ZEB1/SIP1 proteins repress the E-cadherins expression and allow the transformation to metastatic phenotype. The miR-200 family members maintain repressed ZEB1/SIP1 proteins blocking the transformation. The downregulation of these miRNAs arise the ZEB1/SIP1 expression and the following E-cadherin lost resulting in EMT and metastatic phenotype (Eger *et al.*, 2005; Burk *et al.*, 2008; Ma and Weinberg, 2008). In resume we could classify the miRNAs like tumor suppressor miRNAs: miR-17/20, miR-34, miR-335 and miR-220; or like oncogenic miRNAs: miRNA-221/222, miRNA-373, miRNA-520c and miR-10b. But the balance between the oncogenic and suppressor of miRNAs is not the only element which is playing in the posttranscriptional step of the protein regulation process. There are many other elements implicated in the maturation of the proteins. For instance the RNA binding proteins (RBP) can actuate stabilizing the certain mRNA and avoiding the miRNA degradation. This is the case of the RNA binding protein HuR. This protein binds to specific mRNA and does not permit to miRNA to repress them. The HuR presence has been correlated with evidences in augmentation in proliferation and migration capacities. In this case the HuR actuate like an oncogenic element because permit to the malignant proteins to stabilize (Abdelmohsen *et al.*, 2007; Cherry *et al.*, 2006; Gorospe, 2003; Lopez de Silanes *et al.*, 2005). But even it has been reported that this protein is essential during let-7 miRNA actuation joining to the RISC complex, and in this case actuates like a suppressor. It seems that the role of this protein and other RNA binding proteins is different cell to cell (Kim *et al.*, 2009).

Future perspectives

The role that plays the miRNAs during the protein maturation process is not trivial, and the knowledge which is accumulating around these small non-coding fragments it will be very useful for future applications. It has been demonstrated that in some cases the miRNA profiling correlates better than gene profiling with cell differentiation and proliferation (Iorio *et al.*, 2005). This feature opens the opportunity to use the miRNA patterns like evidences of clinico-pathological markers. The possible use like malignancy biomarkers has to be validated with huge statistical studies that can be performed by interdisciplinary groups composed by biologist, physicians, mathematics and informatics. Further studies of the miRNAs in samples that develop resistances to treatments could be another way to explore, because it could be used to apply the personalize medicine, saving the unfruitful treatments. About the usefulness of the miRNAs like tools for therapy the way is longer. Although there are some preclinical studies using miRNAs like therapeutic targets like the case of miR 21 (Si *et al.*, 2006; Zhu *et al.*, 2007; Frankel *et al.*, 2008) or miR 115 (Scott *et al.*, 2007), still it is precipitated to say that the drugs of the future could be designed against these molecules. The multitarget capacity of each miRNA and the specificity of the actuation of each one from cell type to cell type complicate the design of a compound that can actuate upon a miRNA. Each miRNA can actuate suppressing hundreds of mRNA and the repression of an unique miRNA can get multiple results because all around the body in the different tissues; the pathways implicated could be several. For these reason the study of the side effects of compounds designed to actuate in the miRNA activity has to be very precise. Despite the difficulties and multiple still unanswered questions, the study of the miRNAs is a hopeful field that it is growing exponentially in the knowledge year by year, and in the future could be a reality that actually is a perspective.

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