microRNAs: the future for cancer therapy?

Improving cervical cancer treatment

Tim Huitema - 02-07-2010
Medical Oncology and Gynaecological Oncology, UMC Groningen
Supervisor: Dr. Bea Wisman

Abstract

Cervical cancer is the fifth most deadly cancer in humans. More than half of the patients diagnosed with this cancer will die of this disease. After the first treatment 30 percent will develop a recurrence, which has poor survival changes. In this review the possibilities for enhancing the treatment susceptibility will be examined. To address this question this review will look at recent discoveries for the use of microRNA as an interference technique. MicroRNAs are small non-coding RNAs that can inhibit mRNA expression at post-transcriptional level. During cancer a deregulation of miRNA expression levels is detected. Deregulation can affect protein expression, and miRNAs are therefore responsible for disrupted cell properties seen in cancer. The changed miRNA expression profile can be used as a biomarker, as predictor for cancerous tissue, or could be targeted by intervention techniques to restore the disrupted protein levels, or exterminate cancer cells. Some cells in a tumor have stem cell-like properties, they could get insensitive for common treatment, and could grow a whole new tumor. These cells are of special interest for miRNA intervention techniques, because of the developed resistance to conventional therapy. A unique key miRNA in the development of cervical cancer has not yet been found, however a changed miRNA expression of cervical cancer have been found. A combined treatment of conventional therapy and new miRNA intervention technique holds great potential for increasing the treatment susceptibility, and reducing the odds of developing a recurrence.

Index

Introduction

Cancer is responsible for one of the four deaths in the US (1). And it is estimated that one of three people in the developed world will develop cancer during their life time (11). These numbers will rise in de coming years due to the rising life expectancy.
Cancer is caused by mutations in the cell genome. The mutations arise in tumor suppressor and oncogenes (2). These mutations are usually somatic events, however this can also arise in the germ line which leads to heritable mutations. These mutations lead to a changed, and therefore disrupted protein transcription. These alterations change the cell function, and lead to uncontrolled growth, invasion and metastasis of the cells, a condition called cancer. The mutations are usually the result of reactions of the DNA with known carcinogens (3). Commonly known carcinogens are radiation, chemicals and tobacco smoke. These factors reacts with the DNA of the cell and consequently changes the nucleotide sequence of genomic DNA. Also changed epigenetic mechanisms could alter the expression profile of a cells genome, which also leads to disrupted protein transcription.
Another way to get one of the specific DNA modifications to give rise to a cancer is mediated by a virus. When a virus infects the cell, it copies bits of its own DNA or RNA into the hosts genome. The added nucleotide sequence will be translated by the host cell into necessary proteins for survival of the virus. However, it can be that this alteration of the genome leads to a disrupted cell function, and leads to the formation of a cancer.

An example of a virus capable of doing so is the human papillomavirus (HPV) (4). There are over 100 different types of HPV, however type 16 alone is responsible for half of the diagnosed cervical cancers. HPV is sexually transmitted and can infect both man and women. The virus can do harm when it infects the noncornified epithelium tissue of the cervix uteri and can give rise to a state, pre-cancer, where the abnormal growth of cells on the surface of the cervix, cervical dysplasia, occur. This state is divided into three progressing categories, cervical intraepithelial neoplasia (CIN) 1-3 (5). Having a pre-cancer does not necessarily lead to malignant tumor, it is possible for a non-oncogenic HPV to progress to a CIN2 . Therefore, 90% of the HPV infections are harmless, and the symptoms are disappeared within 2 years (6). For 10% of the cases, when the neoplasia is not detected, the infection will grow out into a malignant neoplasm, cervical cancer.

Cervical cancer is the fifth most deadly cancer for women (7). In 2008 of the 529.000 women who got diagnosed with cervical cancer, 52% died of this disease (8). When the cancer is diagnosed to late, the cancer cells might spread out to other organs, such as the abdomen or the lungs. When the tumor is detected a treatment is started. For determination of what treatment is needed, the tumor is categorized from 1 to 4 for severeness. The treatment consist of hysterectomy, radiotherapy and chemotherapy (9). Of the patients diagnosed with an advanced stage cervical cancer 30% will develop a recurrence within 2 years after treatment. The chances of survival after recurrence are poor (10).

Recently discoveries in the oncology field have detected a significant role for epigenetic mechanisms in the oncogenesis . One of the members of these mechanisms are the microRNAs (miRNAs). This are small non-coding RNAs that can inhibit mRNA expression at post-transcriptional level. In this review the possibilities for enhancing the treatment susceptibility for cervical cancer will be examined. To address this question this review will focus on the use of miRNA as an interference technique.

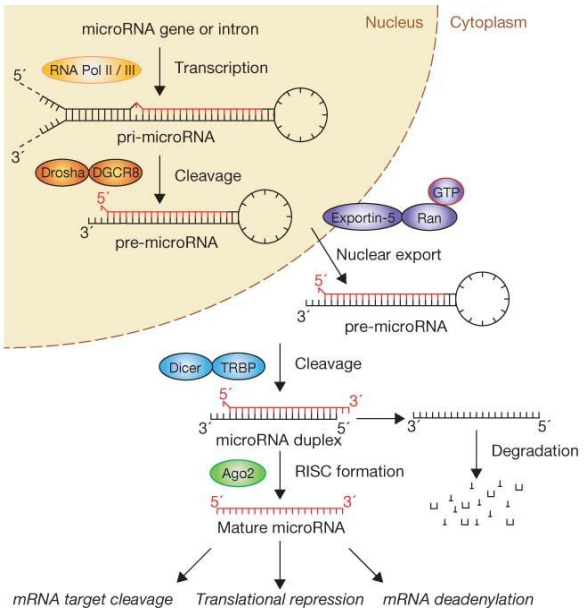
What are miRNAs?

The existence of microRNA was discovered in 1994 through a genetic screen for temporal control of post-embryonic development in *C. elegans* (12). In *C. elegans*, cell lineages have distinct characteristics during 4 different larval stages (L1–L4). It was discovered that the LIN-4 protein abundance, which has to be downregulated for the larva to advance to stage L2, was negatively influenced by a non-protein coding gene, RNA *lin-4.* It lasted until 2000 until a second member of the miRNA family was discovered (13). Nowadays over 100 different miRNAs are discovered in various organisms (14).

miRNA biosynthesis

The transcript coding for miRNA is found in intergenic regions containing clusters of distinct miRNAs or found as a transcript containing both a gene and the miRNA, where the miRNA transcript is found in the intronic region of the gene. The miRNA coded in intergenic regions is transcribed by the RNA polymerase II, which binds at a promoter region on the DNA region, resulting in a primary- or pri-miRNA. The pri-miRNA consists of 100 to 1000 nucleotides containing multiple miRNA stem loops. The pri-miRNA undergoes two sequential processing steps to become a mature miRNA. The first step, which occurs in the nucleus, cleaves the miRNA stem loop from the remainder of the transcript by a member of RNA polymerase III, Drosha. The miRNA spliced out of the introns are bypassing this first step. For the next step the pri-miRNA is transported out of the nucleus by nucleocytoplasmic shuttle Exportin-5 to the Dicer enzyme in the cytoplasm. This enzyme excises the terminal loop, resulting in a mature miRNA duplex of approximately 22 base pairs length (15,16).

From the created miRNA duplex, one stand is incorporated in the RNA-induced silencing complex (RISC), which contains Dicer and proteins of the Argonaute family, to form a miRISC. The other miRNA stand is degraded. The RISC complex delivers the mature miRNA to their mRNA target. Recognition is based upon the complementarily of the miRNA to a specific sequence within the 3` untranslated region of the mRNA (17). When the recognition is complete the cellular function of miRNA takes place. After specific sequence mRNA is recognition, the RISC complex will fully or partially bind. This leads to an inhibition of the protein translation. When the miRNA is fully complementary to the mRNA, Ago proteins catalyze cleavage of the mRNA stand, and therefore silencing of the mRNA. However, protein inhibitions also can take place when the miRNA is only partially complement to the mRNA. The mechanism behind this is not completely resolved, and there is some debate whether the inhibition takes place before or during the protein translation (16). A mechanism that has been proven is the partially complementary binding to mRNA leading to an increased deadenylation of the mRNA (18). That is the increased speed by which the poly-A tail of the mRNA is reduced in length. This leads to an increase in mRNA degradation, thus a reduced protein translation. This biosynthesis is graphically depicted in figure 1.



Figuur 1: miRNA biosynthesis Winter et al. 2009

miRNA and cancer

As described above, miRNAs were first discovered in studies during the development stages of *C. elegans*. Studies in a different model organism *Drosophila melanogaster* revealed a disruption of temporal and spatial expression patterns by miRNAs during development. Blocking the miRNA function with anti-sense molecules during the development of *Drosophila melanogaster* causes clear distinct phenotypes, such as segmentation, development of the nervous system or head involution/dorsal closure (19). Other studies confirmed this importance of miRNAs during the development of *Drosophila melanogaster* (20). When the research was expanded to vertebrates the same results were found. Because of the multi-gene localization of miRNAs the possibility, at that time, to create knockout mice was impossible. However, for miRNAs to function properly, the specific enzyme Dicer is required, as described above. Conditionally knockout of this enzyme during the embryonic phase of mice caused defective branching of lung epithelium. It was found that this defective branching was the result of the over expression of the responsible fibroblast growth factor 10 (21). Several other studies also identified the importance of miRNAs in proliferation, differentiation, apoptosis and development of cells (14). These processes are the ones that are deregulated in cancer cells, and are the processes that cause the high lethality of carcinomas. The involvement of miRNAs during development suggest a major deregulation of miRNAs in cancer.

The role of miRNA during cancer

The first evidence for the involvement for miRNAs in the pathogenesis of cancer came in 2002. In this study it was found that two types of miRNA, miR-15a and miR-16-1, are located on chromosome 13q14. This region is deleted in more than half of B cell chronic lymphocytic leukemias (B-CLL). Consequently, the expression of both miRNAs is downregulated in 68% of the CLL cases(22). During the last years more miRNAs were identified to be deregulated in cancer cells. In most cancer types the deregulation consist of a downregulation of miRNA expression, leading to inhibition of silencing of genes regulate for growth, proliferation or apoptosis, and causing a carcinoma(23). However in some carcinomas miRNA is overexpressed. Normally miRNA act like a protein coding tumor suppressor gene, through silencing the mRNA transcribed from oncogenes. But when overexpressed, the miRNA could also target the tumor suppressor gene, and thus act like an oncogene (24). This can be clarified with an example. Upregulation of miR-125b, a type of miRNA, has an oncogenic growth-stimulatory function in prostate cancer cells. However, it is reported that the same type of miRNA is downregulated in breast cancer, and acts as a tumor suppressor. Similarly, inhibition of miR-21 activity significantly increased the growth of HeLa cervical cancer cells, but resulted in a reduced growth of A549 lung cancer cells. Therefore, the function of a cancerous miRNAs is cell context-dependent (27). This evidence shows that the deregulation of miRNA play a critical role in the pathophysiology of cancer.

One explanation for the changed miRNA expression is a changed genetic profile, caused by chromosomal abnormalities. Of the known miRNA genes, more than half (52,5%) reside in fragile sites, minimal regions of amplification or loss of heterozygosity and common breakpoint regions, all frequently associated with cancer (25). This is confirmed by other studies where overexpression of miR-17-92 is caused by an amplification of its gene locus, whereas a decreased expression of miR-15/16 is due to the deletion of the cluster where this miRNA resides, chromosome 13q14.3 (27). Also the copy-number alteration of miRNAs are highly prevalent with cancer. A study reported that altered genomic loci containing miRNA genes occurred in 37.1% of ovarian cancer, 72.8% of breast cancer and 85.9% melanoma cases (26).

One level where the miRNA silencing in cancer is caused, are epigenetic mechanisms, such as repressive histone modification and CpG island methylation. This was demonstrated by a study where a comprehensive expression profile of miRNAs of bladder tumor cells where taken after a drug treatment with demethylating agents. The expression profiles showed that the agents highly induced the expression of miR-127 (28). In contrast, the locus of let-7a-3 is heavily methylated in normal tissue samples, but is demethylated in some lung cancer samples (29).
An interesting observation is that miRNAs are not only influenced by the epigenetic machinery, but can also interfere in aspects of this of this machinery, through modulation of mechanisms responsible for DNA methylation and histone modifications . This is confirmed by a recent study. In lung cancer upregulation of two key enzymes involved in DNA methylation and down-regulation of miR-29b is found. This miRNA is targeted at these enzymes, and in cancer cells it causes a global DNA hypomethylation. Enforced expression of miR-29b leads to restoration of normal patterns of DNA methylation, and consequently inhibits tumorigenicity (30).

The changed miRNA expression could also be caused at the transcription level. As described above, miRNAs are transcribed by RNA polymerase II. This implies the use of transcription factors, for example, the miR-34 family is a direct transcriptional targets of p53, a tumor suppressor protein. When p53 is activated with adriamycin, a DNA-damaging agent, it induces the expression of miR-34a (31). This transcriptional activation of miRNAs is also demonstrated for Myc, a oncogenic transcription factor (32).

Another level where the miRNA expression could be altered is at the post-transcriptional level. Maturation of miRNAs requires the coordinated processing by the miRNA machinery. For maturation of miRNA the two core RNase III endonucleases, Drosha and subsequently Dicer, are required for post-transcriptional processing. Research is showing that the activity of these two enzymes affect the cellular abundance of miRNAs. Analysis of primary tumors showed an overall downregulation of miRNAs, due to a error in the Drosha processing step. (33) For the Dicer enzyme the same correlation was found. An increase of Dicer expression in prostate cancer was seen at the same time with a global increase of the miRNA expression (34). Also a role for the above mentioned tumor suppressor protein p53 was found. It was found that, in response to DNA damage, p53 enhances the post-transcriptional maturation of several miRNAs with a growth-suppressive function. Where transcriptionally inactive p53 mutants, thus cancerous, interfere with a functional assembly between Drosha complex and p68, leading to attenuation of miRNA processing activity (35).

As described above miRNAs have been of much interest in tumor cell biology because of the role in the initiation and progress of some cancers, where they function as oncogenes or tumor suppressors. In addition, another way in which miRNAs play a role in cancer is more concerned to the treatment of cancer. Expression profiling of miRNA in drug-sensitive and drug-resistant tumor cell lines suggested a role for miRNAs in the development of drug resistance (36). For example, doxyrubicin resistant breast cancer cells exhibit a considerable dysregulation of the miRNAome profile and altered expression of miRNA processing enzymes Dicer and Argonaute 2. These cells also express very high levels of an important mechanism for drug efflux, P-glycoprotein, which is encoded by the mdr-1 gene. The complementary miRNA responsible for this overexpression is caused by the dysfunction of miRNA-451 (37). Further is demonstrated drug metabolizing enzymes and transporters are regulated post-transcriptonally by miRNAs, and, that this post-transcriptional regulation can be induced by drug treatment (38). This is the case in xenograft tumors following gemcitabine treatment. In these tumor cells downregulation of miR-328, involved in the negative regulation of the ABCG2 efflux transporter, is measured. Therefore downregulation of miR-28 leads to overexpression of the transporter, thus multidrug resistance (39).

A last way in which miRNA expression could be altered is mutation. Mutations in the 3'UTR of mRNA, which is target of miRNA, could interfere with the miRNA function. A single nucleotide

polymorphism (SNP), a naturally occurring mutation, near the binding site of miR-24 in the 3'-UTR of the human dihyrofolate reductase (DHFR) gene prevents the miR-24 to recognize this mRNA. And consequently prevents the repression of this protein by miR-24. This results in the overexpression of DHFR and resistance to methotrexate, a cancer treatment drug (40). The same results are discovered in another system; several drug resistant cell lines that overexpress ABCG2, an ABC transporter, were shown to contain truncated 3'-UTRs. The deleted region contain a target binding site for miR-519c, and the absence of this region leads to impairment of miRNA binding, causing overexpression of ABCG2 and drug resistance (41). An interesting observation is that mutations in the 3'-UTR binding site of the mRNA occur at a higher level than in the miRNA sequences themselves. This is probably due to the fact that miRNAs have multiple targets, and therefore mutation of the miRNA genome is likely to lead to more serious consequences to the cell, or organism (42).

The effect of microRNAs on the therapy

microRNAs as biomarkers

One application in the clinic for the miRNAs is to use them as biomarkers, as diagnostic and treatment predicting values. Current research has shown that tumor derived miRNAs are protected from RNases and therefore can be detected in blood and in sputum. This is shown in the detection of non small lung cancer. In these cases the miR-21 is detected in the sputum, and results in a 100% specificity and 70% sensitivity for diagnosis, even in the stage 1 cases (43). Also miRNA markers are found in the blood serum. When miRNAs from human prostate cancer xenografts enter the circulation, they can be used to robustly distinguish xenografted mice from controls. This finding was confirmed in humans where serum levels of miR-141, overexpressed in prostate cancer, could distinguish patients with prostate cancer from healthy controls (44). And again, unlike mRNA, the miRNAs are protected from endogenous RNase activity. Even after days of storage or freeze–thawing cycles. These findings suggest that miRNA biomarkers might be very useful for an early and noninvasive detection of cancer (58).

The use of biomarkers described above, could also be used to discriminate between different tissues, eg. to make the distinction between tumor and benign tissue. Particularly because of the higher stability of miRNA, compared to mRNA. An example, miRNAs could be informative about the prognosis of a cancer. In an expression profile of patients with colon adenocarcinoma, the high expression of miR-21 was a significant predictor for poor survival and poor therapeutic outcome (45). The miRNA can also be used as markers of metastasis, which is the main cause of mortality in patients with solid tumors. Analysis of lymph nodes from metastasis cancer cell lines indentified epigenetic-silenced miRNAs associated with metastasis development. A correlation between hypermethylation of miR-9, miR-34b/c and miR-148a and the occurrence of lymph node metastasis has been found in human tumors (46). The miRNA expression profile was confirmed in another study. The profiles of 43 different tumors from colon, bladder, breast and lung where analyzed and a cancer specific profile for tumor tissue was found. Intriguingly, they also found that the expression profile differs per cancer type, thus are tissue specific. Therefore a simple expression profile from the metastasis could give information about the originating organ of the cancer (47).

Not only can miRNAs give information about the kind of metastasis, miRNAs could also be predictors for the occurrence. In a study tissue samples of 241 patients with hepatocellar carcinoma were analyzed for miRNA expression profiles. From these samples a unique 20-miRNA signature was determined. This signature was significant predictor for survival and recurrence of the cancer. And additionally, this analysis could distinguish between metastatic and non- metastatic tumors (48). Not only the occurrence of specific miRNAs in the serum can be predictive, an absence of specific miRNAs

can be as well. A study reported the loss of expression of miR-126 and miR-335 in the majority of primary breast tumors. Loss of expression of these two miRNAs, associated with reduced proliferation and inhibitions of metastatis respectively, are a poor prediction for metastasis-free survival (49). This absence, compared to wildtype expression, can thus also be detected in the serum or sputum, and therefore be a predictor for metastasis. These recent studies show a promising new way in cancer diagnosis and prognosis, especially due to the occurrence of miRNA in the serum and sputum. Patients could easily be screened for cancer, also as part of a cancer preventive examination.

microRNAs as therapeutics

Because of the property of a single microRNA to influence multiple messengerRNAs, or gene networks, miRNA based cancer therapy can be a great new way to treat cancer, or even cure this disease. General therapeutic strategies should consist of inhibition of the oncogenic miRNAs and replacement of the cancer-associated downregulated miRNAs with miRNA mimetics or viral vector-encoded miRNAs.

For inhibition of miRNA the use of synthetic anti-miRNA oligonucleotides (AMO) has been shown. These miRNAs, modified with an additional 2'-O-mythyl, are effective to endogenous inhibit miRNA. Application of this construct *in vitro*, targeting the onco-miR-21, inhibited glioblastoma and breast cancer cell growth of MCF-7 breast cancer (50). This application is proven *in vivo* to induce a silencing of miR-122, responsible for the cholesterol biosynthesis, for 23 days . And consequently reduces the serum cholesterol levels during this period with 44% (51). An alternative synthetic miRNA can be constructed by adding an extra methylene bridge, connecting the 2'-O and 4'-C atoms of the ribose ring, to form locked nucleic acids (LNA). After administering LNA targeted against the same miR-122 to African green monkeys, a dose-dependent lowering of the plasma cholesterol is measured, without evidence of toxicity (52). However, silencing of only a single miRNA might not be enough for some carcinomas. Therefore development of agents, multiple-target AMOs (MTg-AMO), capable of inhibiting miRNAs simultaneously, is ongoing. For example, an MTg-AMO can be constructed against miR-21, miR-155 and miR-17-5p, all miRNAs frequently overexpressed in tumors. The inhibitory effect of this MTg-AMO is stronger compared with a combination of three single-target AMOs (53). This approach can be useful to make MTg-AMOs designed to target different pathways in the cancer pathogenesis, eg. evading apoptosis, angiogenesis, higher proliferation speed or metastasis.

A novel, alternative, strategy makes use of competitive miRNA inhibitors that can be expressed in cells. This so-called 'miRNA sponges' are vectors expressing artificial miRNA-binding sites that act as sponges for the complementary miRNA, preventing the miRNA to bind to their natural targets (54). An advantage of this approach is that multiple sponges can be combined, and would therefore knockout an entire miRNA family or cluster.

Conversely to knock-out oncogenic miRNAs, strategies to re-express miRNAs might also be an attractive way for cancer treatment. Especially due to the fact that most cancers show a general repression of miRNAs. The following study shows promising results. Cells normally express a high level of miR-26a, however In hepatocellular carcinoma (HCC) expression is reduced. Systemic administration of this miRNA in a mouse model of HCC using adeno-associated virus (AAV) results in inhibition of cancer cell proliferation, induction of tumor-specific apoptosis, and protection from cancer progression. Notably, this finding also revealed that tissue-specific miRNA replacement therapy of miRNAs highly expressed in normal cells is tolerated without evidence of toxicity (55). This result could be of major importance in the clinic. Especially when taken into account that there are more cases where one miRNA is powerful enough to have a dramatic repression of the tumor progression. For example in the treatment of highly metastatic carcinomas. It was found that miR-31

expression is altered in metastasis of breast cancer. Downregulation causes, even in otherwise-nonaggressive breast cancer, cells to metastasize (56). The metastasis was caused by the expression alteration of this one miRNA alone. This could be of major clinical importance to re-express this miRNA, and therefore inhibit the metastasis.

The results of these are promising for use in the clinic, especially when used complementary to traditional anticancer treatments. As described miRNA can effect efflux-mechanism and therefore increasing the drug resistance of cancer cells. However, these mechanisms can be targeted with anti-miR-21 AMOs and consequently increase the susceptibility of colangiocarcinoma cells to the chemotherapy drug gemcitabine (39). This can also been done with restoration of miR-200c in endometrial, breast and ovarian cancer cell lines. Leading to an increase in the sensitivity to microtubule-targeting agents by 85% (57). For further research it would interesting to profile the miRNA differences between the resistant and sensitive cancer cells to identify miRNAs that are involved in the resistant mechanism or pathways, which can then be used to resensitize tumors with miRNAs to improve treatment (51).

microRNA and the cancer stemcell

The cancer stem cell

In the section above the possibility for a combined miRNA and convention cancer treatment is suggested. While this approach might hold great potential, new discoveries suggest a slightly different combination of therapies for maximizing effectively.

Stem cells have the characteristic to be self-renewal and can undergo multi-linage differentiation (59). These cells are found in numerous tissues of the body and fulfill a key role in the tissue development, repair and replacement. Studies suggested an important role for miRNAs in the maintenance of the stem cell function. The expression of certain levels of miRNA are different between stem cells and other normal tissues (60). The importance of this distinction for the stem cell function was proven *in vivo*. When the Dicer-enzyme, important for miRNA biogenesis, was knocked out in mice not only lead this to disrupted growth and death in early development, but also to a depletion of the numbers of stem cells (61). This was confirmed by knock-out study in *Drosophila melanogaster*, mutant Dicer caused delay in the G1 to S transition, therefore reducing the stem cell proliferation speed (62). This findings implicate a important role for miRNAs for stem cell function.

In solid tumors researchers found cells who are displaying a different cell surface markers (63). When isolated, these cells could give rise to a new tumor displaying the same heterogeneity of cell surface markers of the tumor tissue isolated from (64). These cancer cells with stem cell-like properties gave rise to the stem cell hypotheses; tumor-initiating cells that proliferate through their unique self-renewal ability (63). Recent research has discovered more proof for this hypotheses, cancer stem cells has been identified in solid tumors from brain, liver, breast, pancreas and colon cancer tissue (65).

Role of cancer stem cell in treatment

Common anticancer treatment is directed predominantly at the bulk cancer tissue. The effect of this treatment has often limited efficacy, the cancer tissue develops resistance against drug treatment and/or radiation treatment. This resistance consists of increased recognition and repair of DNA damage, reduced functioning of the apoptotic pathways, and increased intracellular drug clearance through increased expression of drug efflux ABC transporters (66). There is evidence that

conventional therapy fail to exterminate a small sub-set of cells able to initiates and perpetuates tumorigenesis; the cancer stem cell population. For example, cancer stem cell radio treatment resistance is shown in human leukemias, in malignant melanoma, and in brain, breast, pancreatic, and colorectal cancers (65).
The existence of a specific cell type, the cancer stem cell, which can undermine treatment through the development of drug and radio resistance, could be an important target for the described miRNA treatment. To cure a patient from cancer, all the malignant cells have to be eradicated. Conventional therapy can be used for treating the bulk, non-cancer stem cells, and miRNA therapy for cancer stem cells, who are responsible for developing radio- and chemo-resistance. Combining the miRNA therapy with conventional therapy might have a great potential for increasing treatment efficiency.

microRNAs and cervical cancer

Now that we discussed the current advances in cancer treatment thought the use of miRNAs, the possibilities for improvement the diagnoses and treatment of cervical cancer specific using miRNA interferece will be discussed. However, the number of research published for unique cervical miRNA expression profiles is low, and a proven key miRNA alteration has not (yet) been found. Therefore, an overview of recent studies will be presented.

Infection of cervical cell with HPV leads to an alteration of DNA with the papillomaviral oncoprotein E6. In cervical cancer tissue, expression of miR-34a is reduced. As described, research has shown a transcriptional effect of the tumor suppressor p53 on miR-34a. Expression of viral E6 has a destabilizing effect on p53, which results in increased cell proliferation. Knockout of viral E6 expression in cervical cancer cell lines leads to an increased expression of p53 and miR-34a. Over-expression of miR-34a in cervical lead to a reduction in cell growth and partially apoptosis (67). This provides evidence for an oncoprotein, E6, to regulate cellular miRNA expression. Another study also identifies miRNA expression alteration by an oncoprotein from HPV. Expression of viral E6 resulted also in a reduced miR-218 expression. And conversely, RNA interference in the E6 oncogene increased the miR-218 expression (68). Therefore, this miRNA is also regulated by an oncoprotein.

While the above described studies aim at unraveling the mechanisms behind viral regulation of miRNA, more recent studies are interested in creating a miRNA based signature for the prediction of cervical cancer survival, using modern high throughput sequencing. After an analysis of 102 cervical cancer samples, two miRNAs, miR-200a and miR-9, are revealed that could predict patient survival. Especially miR-200a is likely to affect the metastatic potential, because of the role in controlling genes for cell motility (69). Another study use high throughput sequencing to analyze 58 miRNA samples prepared from 29 pairs of cervical cancer tissues and matched normal tissues. This lead to the discovery of 67 miRNAs that were differentially-expressed between the tumor and normal samples (70).

There is also another approach to find a key miRNA. This study started from the already known involvement of miR-21 in a wide variety of cancers, including cervical cancer. In this study the effects of this miRNA in cervical cancer were examined. It is shown that inhibition of miR-21 in HeLa cervical cancer cells caused suppression of cell proliferation, and up-regulated the expression of the tumor suppressor gene programmed cell death 4. Therefore suggesting that miR-21 act as an oncogene in the cellular processes of cervical cancer (71).

Discussion

Therapies based on miRNA interference might have great potential for increasing the radio- and chemo sensitivity of patients diagnosed with cervical cancer. The most viable way to increase the sensitivity is through a combined treatment. Whereas the conventional therapy should be directed at the bulk, non-stem cell like, cancer cells. The therapy is a good solution for this purpose, chemo- and radio therapy can easily provoke a lot of cellular damage in a large amount of malignant cells, leading to clearance of these cells. The new miRNA intervention technique should be directed at a small population of cells, the cells with stem cell-like properties, the cancer stem cell. These are the cells that start and maintain tumor genesis. Conventional therapy sometimes fails to eradicate these cells, because of the drug resistance development, leading to the re-growth of drug-resistant new tumor tissue. Therefore a therapy with a combined treatment can greatly improve the susceptibility of cancer cells for treatment, thus reducing the odds of developing a recurrence.

Another function of miRNA could be the use as biomarkers. Traces of miRNA could be detected in the blood or in the sputum, therefore an alteration of these levels might be an indication for cancerous tissue. The great potential of miRNA as biomarkers lies in the usage as a non-invasive screening technique. Women could be screened on a regular basis, and would greatly improve the early detection of cervical cancer, thus increasing the survival rate. An easy detection could especially be beneficial for countries, mostly in un-developed world, where a national screening program is nonexistent nowadays. An possible advantage of using miRNA as biomarkers is that the different cancer types, e.g. lung or cervical, all have a different miRNA expression profiles. Therefore, the changed miRNA levels measured in blood or sputum might directly show in which organ the tumor is originating.

For cervical cancer, a lot of research has to be done on specific miRNA changes during cancer. Some have been identified, but the evidence is not conclusive. Therefore, more research should been preformed. For cancer in general, a lot of obstacles has to be cleared to use the miRNA interference technique in the clinic. First, conclusive miRNA profiling must be done between cancer (stem) cells and healthy tissue, in order to distinguish the two. Second, key miRNAs should be identified in cancerous tissue. And third, a way to deliver the miRNAs, or silencing compounds, exclusively to the cancer stem cells should be invented. Targeting healthy stem cells, especially on top of the overall cellular damage caused by conventional therapy, can lead to serious complications.

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