PROSTATE CANCER AND INFLAMMATION: THE ROLE OF miRNAs

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ABSTRACT

Approximately 15-20% of all human cancers are assumed to be a result of infection and chronic inflammation due to a constant supply of cytokines and reactive oxygen species, giving rise to genomic instability and a subsequent tumour development. In recent years, chronic inflammation has also been hypothesised to influence prostate carcinogenesis, since both acute and chronic inflammation is commonly seen in prostatic tissues. The signalling pathways involved in the immune response and tumour development are overlapping with each other, and it has been proposed that miRNAs are a possible link between the two processes. In this review, we are describing some of the miRNAs which could constitute a conceivable link between inflammation and prostate cancer.

Keywords: Prostate cancer, inflammation, microRNAs.

PROSTATE CANCER

Prostate cancer (PCa) is the most common malignancy among men in Western society. In 2012, almost 360,000 new cases of PCa were diagnosed in the European Union, and 71,000 men died from the disease.[1] Even though PCa is a very common disease, the aetiology is largely unknown. The most established risk factors are family history, age, and African-American ethnicity, although chronic inflammation and infection have also been suggested to play a role in prostate carcinogenesis.[2]

INFLAMMATION-RELATED CANCER

Inflammation was linked to cancer more than a century ago by Rudolf Virchow who observed inflammatory cells in tumour specimens and found that tumours often developed in close vicinity to chronic inflammation.[3] The inflammation could not be exclusively explained as an anti-tumour immune response since it is often scattered throughout an entire organ and it is also commonly seen in pre-lesions to cancer. Today, approximately 15-20% of all human cancers in adults are suggested to result from infection and chronic inflammation.[4] Classic examples of malignancies where inflammation are considered a risk factor are colon cancer arising in individuals with inflammatory bowel disease, and gastric cancer caused by *Helicobacter pylori* infections.[5]

Inflammation is a process that involves both an innate and adaptive immune response following infection or injury. The innate immune system initiates the inflammatory response by producing a large number of cytokines, reactive oxygen (ROS), and nitrogen species (RNS).[6] This process is essential, not only to eliminate pathogens and repair tissue damage, but also to activate the adaptive immune response. Even though inflammation acts as a host defence and usually is a self-limiting process, failure leading to inadequate resolution of inflammatory responses may be pathologically conductive. Chronic inflammation has been linked to tumour promotion and progression by several mechanisms, including increased cell proliferation, enhanced

angiogenesis, and evasion from apoptosis. A constant supply of cytokines, ROS, and RNS in a microenvironment with sustained inflammation may, over time, give rise to genomic instability and subsequent tumour development.[7]

MicroRNAs

MicroRNAs (miRNAs) are small non-coding RNA molecules with a size of 18-24 nt, whose function is to post-transcriptionally regulate genes involved in a wide range of biological processes, such as differentiation, apoptosis, and inflammation.[8,9] Studying the effect of miRNAs in the human genome is complicated due to the fact that one miRNA could regulate the expression of several target genes, and one gene could be regulated by several miRNAs. Furthermore, different miRNAs are expressed in different cell types, and the miRNAs expressed in one cell type could have expression levels that differ up to 1,000-fold across the differentiation stages of that cell.[10] Even though the expression of a miRNA does not change within a cell, it could have different functions between the different differentiation stages within that cell.

Several large-scale studies investigating the miRNA expression patterns in PCa have been performed to date, although the results from these studies are inconclusive with some studies reporting miRNAs to be up-regulated while others report them as down-regulated or not deregulated at all.[11-17] This inconsistency between study results could be due to the study material and method used, but it could also be due to the heterogeneous nature of PCa and tumour development or the complexity of the miRNA system.

miRNAs, INFLAMMATION AND PROSTATE CANCER

It is now well-known that miRNAs are involved in almost all inflammatory responses and that they have a significant impact on the magnitude of the inflammatory response. This is accomplished by influencing the development of inflammatory cells, establishing the level of immune cell function and cytokine production, as well as responding when the immune system encounters pathogens. By activation and repression of multiple miRNAs, the capacity of the immune system is properly balanced, creating a fine-tuned system.[18] A properly adjusted miRNA expression results in a transient inflammatory response that clears the infection without causing any damage to the host tissue. Deregulation of miRNA expression could result in either an immunodeficiency or a hyperactive response to infection, which could be extremely harmful. A constant deregulation of miRNA expression could also lead to a chronic inflammatory state. There are a vast number of miRNAs playing crucial roles in the inflammatory response, of which miR-146a, miR-21 and miR-155 are among the most well-described in the literature today. A summary of the regulatory functions of these miRNAs on the inflammatory response can be seen in Figure 1.

miR-146a

MicroRNA-146a (miR-146a) has been proposed to play a role in regulating toll-like receptor (TLR) signalling in response to bacterial pathogens by preventing excessive inflammation. Thus, the role of miR-146a is to dampen the production of pro-inflammatory mediators such as IL-6 and TNF-α, serving as a negative regulator of the immune system.[19,20] miR-146a is activated in immune cells through cell-surface TLRs (TLR-2, -4 and -5) sensing bacterial pathogens or in response to the pro-inflammatory cytokines IL-1β and TNF-α.[21] This up-regulation occurs when NF-κB binds and trans-activates the gene promoter of miR-146a, leading to activation and subsequent repression of miR-146a target genes, TRAF6 and IRAK1, involved in the TLR/NF-κB pathway.[19-21] Other validated target genes of miR-146a are cyclooxygenase 2 (COX-2) and IL-6, and a down-regulation of miR-146a leads to an increased expression of both of these genes.[22] COX-2 is a key enzyme in the conversion of arachidonic acid to prostaglandins (PGs), and the expression of COX-2 has been found to be elevated in a variety of cancers, for example, breast and prostate. High levels of COX-2 lead to an increased synthesis of PGs, which in turn is believed to contribute to cancer pathogenesis, mainly due to their effect on cell proliferation, angiogenesis, and apoptosis. There are only a few reports on the expression of miR-146a in PCa, stating that a reduced expression of miR-146a is associated with PCa.[14,23]

miR-21

MicroRNA-21 (miR-21) is another miRNA which is induced by NF-κB during TLR-4 signalling. Once activated, this miRNA targets and represses the

pro-inflammatory PDCD4, enhances the production of the anti-inflammatory cytokine IL-10, as well as decreasing the pro-inflammatory activity of NF-κB, thus constituting another negative feedback loop that mutes the immune response.[24] Enhanced levels of IL-10 have been suggested to have an impact on anti-tumour immunity, since IL-10 together with TGF-β are able to expand the population of regulatory T cells (Tregs), which have a suppressive function on CD4/CD8 effector T-lymphocytes, thereby promoting tumour growth. miR-21 has been validated as an oncogene and it is one of the most frequently up-regulated miRNAs in solid tumours including PCa where it promotes survival, anchorage-independent growth, and proliferation.[14,17,25]

miR-155

MicroRNA-155 (miR-155) is one of the best characterised miRNAs to date and it has been implicated to play a role in both the innate and adaptive immune system, as well as in the development of immune cells. This is a pro-inflammatory miRNA which regulates the immune system through the help of a wide range of inflammatory factors such as cytokines and components of the NF-κB pathway. miR-155 targets and down-regulates SHIP1 and SOCS1, leading to an increased activation of Akt and IFN pathways, thus mediating cell survival, growth, and migration.[26,27] miR-155 is also induced through TLRs sensing bacterial and viral pathogens, by TNF-α and through NOD2 sensing bacterial peptidoglycan, suggesting that it is a key player in the immune response towards a broad range of inflammatory mediators.[28,29] Elevated levels of miR-155 lead to increased levels of pro-inflammatory factors, but it has also been shown to lead to an enhanced rate of spontaneous mutations, since miR-155 also targets components of the DNA mismatch repair machinery. If an inflammation becomes chronic, the rate of spontaneous mutations could increase further, and together with a simultaneously miR-155 driven suppression of tumour suppressor genes such as TP53BP1, this could shorten the series of steps required for carcinogenesis.[30,31]

miR-155 has been found to be deregulated in several types of cancer, such as breast cancer[32] and pancreatic cancer,[33] although to our knowledge there are no reports on deregulation in PCa tissues but unpublished results from our group show that miR-155 are up-regulated in the PCa cell line LNCaP (Carlsson et al., Unpublished results).

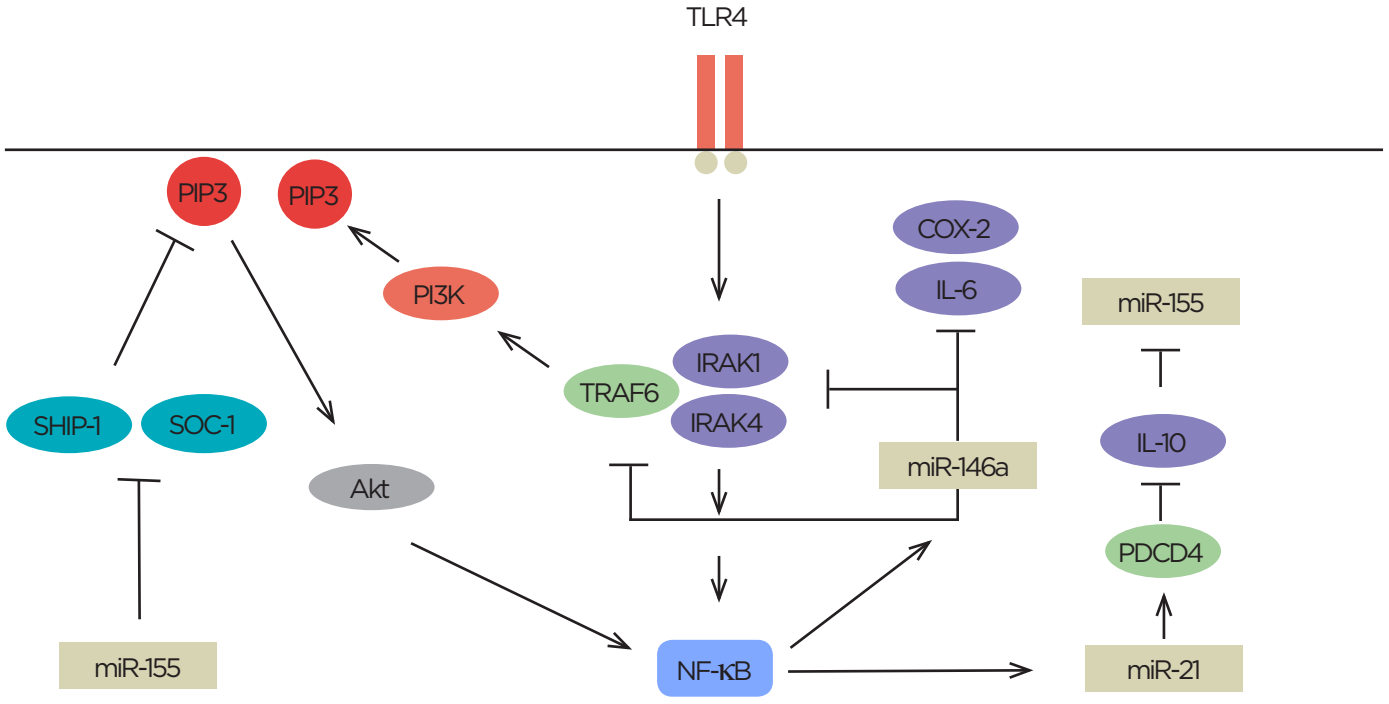

Figure 1. A schematic overview of the regulatory effects of miR-146a, miR-155 and miR-21 on the inflammatory response.

CONCLUDING REMARKS

Nowadays it is well-known that the signalling pathways that are involved in the immune response and inflammation are overlapping with the pathways involved in tumour development, and it has been suggested that it is the expression of miRNAs that links these two processes together.[34] Although, exactly how miRNAs link inflammation and tumour development together, is currently unknown. Thus, the main question is; is it a deregulation of miRNAs (such as miR-155 overexpression and down-regulation of miR-146a) that leads to a chronic inflammation, creating a microenvironment that favours tumour development, or is it a chronic inflammation that leads to a deregulated miRNA expression, which in turn could favour a tumour development?

When performing a literature review, three interesting miRNAs in the context of inflammation and cancer emerge, miR-146a, miR-21 and miR-155. The most interesting of these is miR-146a, which has been found to be deregulated in previous miRNA expression studies in PCa. The role of miR-146a is to dampen the production of pro-inflammatory mediators, a down-regulation of miR-146a leads to increased levels of these mediators, which could result in a chronic inflammation and thus a tumour-stimulating microenvironment. In addition, it could also result in a constant activation of NF-κB, which has been found in several cancers including PCa. NF-κB might be linked to tumour development through induction of pro-inflammatory cytokines, such as IL-6, TNF-α, and COX-2, and may also contribute to genomic instability by promoting release of ROS and RNS. Consequently, a down-regulation of miRNA-146 may have an essential role in early-stage cancer development. If this down-regulation of miR-146a is found together with an elevated level of miR-155, this could increase the risk for tumour development since increased miR-155 levels lead to an enhanced production of pro-inflammatory mediators as well as an enhanced mutation rate. Furthermore, deregulated miRNAs may also participate in later stages of the prostate carcinogenesis. The up-regulation of miR-21 seen in PCa and the subsequently increased levels of IL-10 are suggested to have an impact on the anti-tumour immunity. Evidence has been provided showing that IL-10 and TGF-β are able to expand the Treg population and thereby aid tumour growth. Our group has recently found that men with greater numbers of Treg in their prostate tumour environment have an increased risk of dying of PCa.[35]

Another aspect of the link between miRNAs, inflammation and cancer is infectious agents, and even though host miRNAs are important in the response against infectious pathogens, there are also some pathogens that benefit from the host's miRNAs in their pathogenesis. One such example is Marek's disease, where host miR-155 is essential for the oncogenic potential of the pathogen, thus demonstrating a link between inflammation and cancer following infection.[20] The expression of miRNAs have also been established as a link between infection and the development of cancer in a recent study performed on gastric cancer, where patients with a polymorphism in miR-146a in combination with a Helicobacter pylori infection had a higher risk for developing cancer.[36] It is believed that this polymorphism in the precursor of miR-146a could reduce the production of mature miR-146a, thus leading to a modified inflammatory process where the patient becomes more vulnerable to infections. The same polymorphism has also been found in patients with PCa and was then associated with a higher risk of developing PCa.[37] Even though there have not been any studies investigating whether the risk for PCa is further increased if the polymorphism is found in combination with an infectious agent, it could be hypothesised that this is the case. It is important to keep in mind that not all chronic inflammation in the prostate leads to tumour development. However, in the cases where inflammation does lead to tumour development, it could be hypothesised that it is genetic changes in miRNAs, such as a polymorphism in miR-146a, which predispose these men to PCa development caused by a chronic inflammation. To date, there are no specific infectious pathogens associated with PCa, although the results from other studies suggests that miRNAs could be an important part of any infectious agent's mechanism of infection and its oncogenic potential in PCa as well.

Based on the literature, we suggest that deregulation of inflammatory associated miRNAs, such as miR-146a, miR-21 and miR-155, have the capacity to influence both PCa initiation and progression. To our knowledge, there are no

miRNA expression studies published where the PCa cases studied had a confirmed inflammation (neither acute nor chronic). In order to validate the hypothesised connection between miRNAs, inflammation, and PCa, more studies need to be performed with the specific purpose to study this link.

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