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COMMENTARY

Targeting the oncogenic role of miRNA in human cancer using naturally occurring compounds

Stuart A Rushworth

School of Pharmacy, University of East Anglia, Norwich, UK

Correspondence
Stuart A Rushworth, School of Pharmacy, University of East Anglia, Norwich NR4 7TJ, UK.
Email: s.rushworth@uea.ac.uk

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Micro-RNAs (miRNAs) are small RNA molecules that regulate the expression of genes involved in development, growth, proliferation and apoptosis. In cancer several miRNAs have been functionally classified as oncogenes or tumour suppressers or act to regulate transcription factors, like nuclear factor kappa B and NF-E2–related factor 2, in cancers such as leukaemia, breast and colorectal. Therefore, it has been proposed that manipulating miRNA regulation may be a novel avenue for developing efficient therapies against cancer. In this issue, Li and colleagues describe a novel way of targeting miRNA, by using a naturally occurring anti-cancer compound found in mistletoe which they showed to down-regulate miR-135a&b, which target the 3'untranslated region of adenomatous polyposis coli gene, the inactivation of which is a major initiating event in colorectal tumourigenesis. This commentary aims to discuss the regulatory mechanisms of miRNA synthesis and the potential outcomes for using naturally occurring compounds antioxidants or cellular antioxidant pathways to target miRNA for therapeutic intervention.

LINKED ARTICLE
This article is a commentary on Li *et al.*, pp. 349–364 of this issue. To view this paper visit http://dx.doi.org/10.1111/j.1476-5381.2010.01042.x

Abbreviations
miRNA, microRNA; Nrf2, NF-E2–related factor 2

Introduction

To date, a number of studies have shown the ability of individual microRNAs (miRNAs) to regulate oncogene and tumour suppressor gene expression and others have shown that miRNA gene loss or mutation can contribute to tumourgenesis. Therefore, understanding the regulatory processing of miRNA is fundamental to determining potential targets for activation or deactivation. Here I will review the regulatory mechanism of miRNA biogenesis and discuss the potential of naturally occurring compounds as new approaches which block the function or increase expression of miRNA, resulting in the inhibition of any given oncogenic effect. The accomplishments in this field of research have revealed a potential for miRNA to be used as a clinical target for therapy.

Regulation of miRNA synthesis

Mature miRNAs are very short, only about 22 nucleotides. In contrast, primary miRNAs (pri-miRNAs) are usually long transcripts ranging from hundreds to thousands of nucleotides in length, which are initially transcribed by RNA polymerase II in the nucleus as long pri-miRNA transcripts. These transcripts need to be processed through three cleavage steps prior to their activation in gene regulation. In the first step, a pri-miRNA is cleaved in the nucleus into an intermediate called the precursor miRNA (pre-miRNA). In the second step, the pre-miRNA is exported to the cytoplasm, where it is cleaved into a miRNA duplex. In the final step, the miRNA duplex is then incorporated into an effector complex called the miRNA-induced silencing complex to give a single-stranded active miRNA.

The recently established importance of miRNA in cellular and tissue homeostasis implicates that pri-miRNA transcription and processing needs to be tightly regulated. Pri-miRNA is transcribed as primary transcripts that require subsequent processing to generate a functionally mature miRNA. In human cancer, pre-transcriptional regulation of miRNA expression can be affected by mutations or epigenetic inactivation of the miRNA promoter region for example, specific activation of microRNA-127, which targets the down-regulation of the proto-oncogene BCL6, by

chromatin-modifying drugs was shown in human cancer cells (Saito *et al*., 2006). More recently, a number of transcription factors that regulate gene expression in human cancer have been shown to regulate expression of cancer related miRNA.

Once the pri-miRNA has been transcribed it is processed in the nucleus by the microprocessor complex which contains the enzyme Drosha, partnering with the double-stranded RNA binding protein, DGCR8. The Drosha-DGCR8 complex converts pri-miRNAs into shorter, stem-loop structured dsRNAs of 70–80 nucleotides called pre-miRNAs, with the mature miRNA included in this sequence. After successful cleavage, the pre-miRNA is bound by exportin-5 and exported from the nucleus. In the cytoplasm, pre-miRNA undergoes the next step of processing mediated by Dicer to produce the mature miRNA.

Both Drosha and Dicer are general factors that non-specifically control miRNA processing, and thus their activity regulates the cellular abundance of all miRNAs. In a number of studies variable levels of both Dicer and Drosha have been linked to poor prognostic outcomes in various cancers. For example, in one study more than 39% of ovarian tumours had decreased Dicer and Drosha mRNA levels and these expression levels were associated with poor patient outcome. These poor survival outcomes were also validated in independent data sets of patients with ovarian, breast and lung carcinoma (Merritt *et al*., 2008). Others have examined the role of RNAi machinery expression in tumour types such as breast, neuroblastoma and lung cancer. Moreover, a recent study shows that in human breast cancer, high levels of miR-103/107, the target for which is Dicer, are associated with metastasis and poor outcome and that inhibition of miR-103/107 prevents migration and metastasis of malignant cells (Martello *et al*., 2010). In contrast to these studies, Chiosea reported (2006) that high Dicer expression was a poor prognostic factor in patients with prostate carcinoma (Chiosea *et al*., 2006). The differences in these reports suggest that RNAi regulatory processes may be tumour-specific and targeting the miRNA regulatory machinery is a realistic option in these tumours

Of the various levels of regulation both pre- and post-transcriptional regulations are generally seen as less miRNA-specific, whereas regulation at the transcriptional level offers a higher degree of specificity as transcription factors are presumably involved in the development- and cell-specific regulation of distinct miRNAs. However, all three regulatory mechanisms present potential targets for the activation or deactivation of miRNA function.

Therapeutics to target miRNA

Many studies have indicated that miRNAs can serve as novel therapeutic targets for cancer. For miRNAs with oncogenic capabilities, potential therapies include anti-miRNA oligonucleotides, microRNA sponges, miRNA masking, and small molecule inhibitor. For tumour suppressor miRNAs, restoring suppressor miRNAs by forced expression of those miRNAs may be a useful strategy. However, a number of questions do need to be addressed before such therapy becomes available for the treatment of cancer. These include 'What is the efficacy and safety in preclinical models?', 'What is the stability and cell penetration?', and finally, 'What is toxicity to surrounding non-cancerous tissue?'.

Another strategy, which needs to be evaluated, is the use of naturally occurring anti-cancer compounds or antioxidants. In this issue, Li *et al*. (2010) demonstrate an alternative, less complicated and easily administered method for the treatment of cancer by targeting miRNA. This method uses the naturally occurring anti-cancer compounds found in mistletoe (CM-1). The use of this compound showed a significant down-regulation of miR-135a&b, which target the 3′ untranslated region of adenomatous polyposis coli gene, the inactivation of which is a major initiating event in colorectal tumourigenesis. More surprising was the finding that the down-regulation of miR-135a&b induced by CM-1 were not due to the suppression of their gene transcription, but rather to the specific degradation of their pre-miRNAs. That is, the pre-miRNA degradation induced by CM-1 hinders miRNA processing step and reduces mature miR-135a&b formation.

This is not the first time it has been shown that naturally occurring compounds can regulate miRNA expression in human cancer cells. Tsang and Kwok (2010) recently reported that epigallocatechin gallate (EGCG), which is a major type of green tea polyphenol, can up-regulate miR-16 which targets the anti-apoptotic protein Bcl-2. Furthermore, this group showed that EGCG treatment induced apoptosis and down-regulated Bcl-2 in HepG2 cells (Tsang and Kwok, 2010). Resveratrol is another natural antioxidant with anti-cancer properties that is currently at the stage of preclinical studies for human cancer prevention. With regards to the effects of resvertrol on miRNA expression, research by Tili *et al*. (2010) have shown that in human THP-1 monocytic cells resveratrol up-regulates miR-663, a microRNA potentially targeting multiple genes implicated in human cancer (Tili *et al*., 2010). They showed that resveratrol impairs the up-regulation of miR-155 in a miR-663-dependent manner, and given that miR-155 is up-regulated in many cancers, these results suggest that manipulating miR-663 levels may help to optimize the use of resveratrol as an anti-cancer agent against malignancies associated with high levels of miR-155.

Another possible avenue, which has been suggested, is to target the regulation of miRNA through the cellular antioxidant pathways that are regulated in the main by the transcription factor NF-E2–related factor 2 (Nrf2). The Nrf2 pathway has been shown to be activated by dietary antioxidants such as EGCG, sulphoraphane and resveratrol. However, a recent publication shows that miR-144 down-regulates Nrf2 mRNA levels and subsequent hydrogen peroxide-induced antioxidant and detoxification gene expression in the human acute myeloid leukaemia cell line K562 (Sangokoya *et al*., 2010). Mir-144, which has also been shown to be involved in erythropoiesis, is also found to be down-regulated in acute myeloid leukaemia. Furthermore, it has been shown that Nrf2 is highly expressed in human acute myeloid leukaemia cells and that this can lead to protection from certain chemotherapeutic drugs (Rushworth and MacEwan, 2008). Together, these studies suggest that using antioxidants to regulate miRNA through Nrf2 regulation in human cancer needs to be investigated further.

Conclusion

From the standpoint of developing novel therapies targeting miRNAs together with the number of issues still apparent with using small molecular inhibitors, the study published in this issue by Li *et al*. demonstrates that we should be looking to ascertain the usefulness of natural compounds as a bridge to targeting oncogenic miRNA.

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