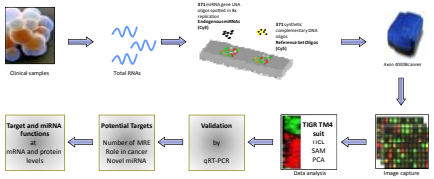
Co-regulation of the tumour suppressor gene programmed cell death 4 (PDCD4) by miR-21 and miR-499 in head and neck cancer

Xiaoying Zhang [1, 2], Barbara Rose [1, 2], Rosetta Martiniello-Wilks[3] and Nham Tran [1,2, 3]

Department of Infectious Diseases and Immunology, University of Sydney[1], The Sydney Head and Neck Cancer Institute, Sydney Cancer Centre, Royal Prince Alfred Hospital [2], NSW, 2006 and the Translational Cancer Research Group, Dept Medical & Molecular Biosciences ,University of Technology Sydney[3] , NSW, 2007, Australia

Method

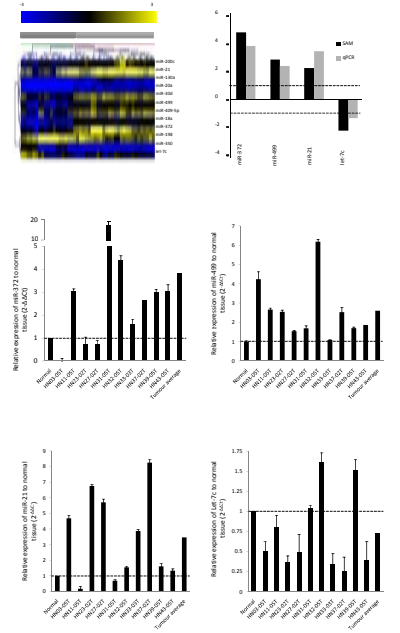


Total: 8 paired, 7 unpaired fresh tissue samples (total samples: 23) were arrayed in duplicate and 12 paired fresh tissue samples (total 24) were used in qPCR procedures and all samples were pathologically approved.

Introduction

Head and neck cancer is the 8th most common cancer but little is know about the mechanism(s) or genes driving this malignancy. This study will explore the expression and function of microRNAs (miRNAs) in tonsillar cancer (a subset head and neck cancer). Matching tumour and adjacent normal tissues were arrayed using a miRNA LNA probe library. MiR-21 and miR-499 were significantly up-regulated in tonsillar cancer and both miRNAs were predicted to regulate the tumour suppressor PDCD4. Moreover, tonsillar tumours with up-regulated miR-21 and miR-499 showed low levels of PDCD4 as detected by qPCR and immunohistochemistry. The inhibition of miR-21 by LNA anti-sense in head and neck cancer cell lines up-regulated PDCD4 at both RNA and protein levels. Interestingly, the initial down-regulation of PDCD4 was mediated by miR-21, but for sustaining repression, miR-499 was needed. The over-expression of miR-21between 0 to 48 hours showed a down-regulation of PDCD4 but this regulation was lost at 72 hours. In contrast, over-expression of miR-499 had no effect on PDCD4 expression between 0 and 48 hours and was only evident starting at 48 hours. This result suggests a co-regulatory mechanism for PDCD4.

Results

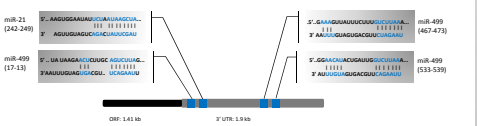


Top figures: *Identification of differentially expressed genes by using two class SAM analysis and then validated by TaqMan (ABI) qPCR. The range of expression values is from -3.0 to 0 to 3.0 fold and miRNA genes shown in yellow and blue represent up-regulated and down-regulated, respectively.*

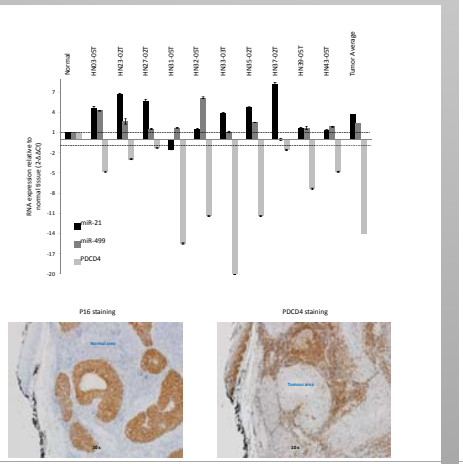
Middle figures: *Validation of miR-372 and miR-499 using qPCR. All tonsil SCC samples demonstrated a increase in miR-499*

Bottom figures: *Validation of miR-21 and let-7c using qPCR. 80% tonsil SCC samples demonstrated a increase in miR-21*

Results



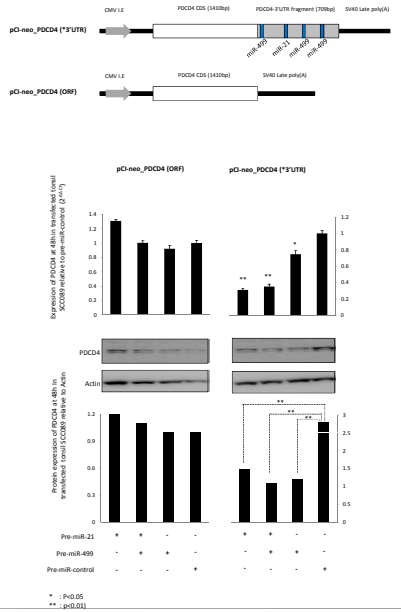
miR-21 and miR-499 have complementary sites on 3'UTR of PDCD4



Top Figure: *Relative expression levels of miR-21, miR-499 and PDCD4 were measured from 10 independent matched normal and tumour tonsil samples*

Bottom Figure: *Tonsil tumours with up-regulated miR-21 and miR-499 showed low levels of PDCD4 as detected by immunohistochemistry.*

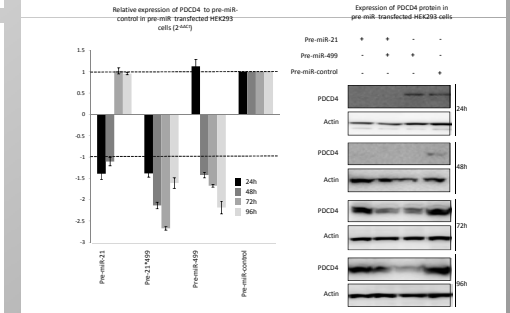
Results



Top Figure: *Plasmid constructs for the over-expression of PDCD4 with and without the 3'UTR.*

Bottom Figures: *PDCD4 over-expression in tonsil SCC cells was negatively regulated by miR-21 and miR-499 at both the mRNA and protein levels*

Results



Inhibition of miR-21 by LNA anti-sense in head and neck cancer cell lines. The reduction of miR-21 was marked by a increase in PDCD4 at both mRNA and protein levels.

Left figure: *Initial down-regulation of PDCD4 mRNA was mediated by miR-21, up to 48 hours post transfection. Sustain repression of PDCD4 was then mediated by miR-499.*

Right Figure: *Over-expression of miR-21 between 0 to 48hours showed a down-regulation of PDCD4 protein but this regulation was lost at 72 hours. In contrast, over-expression of miR-499 had no effect on PDCD4 expression at 24 hours and was only evident at 48hours. This suggests a miRNA co-regulatory mechanism for expression of PDCD4*

Conclusion

This study has identified differentially expressed miRNAs in tonsil cancers and provides new evidence for the co-regulation of the oncogene PDCD4 by miR-21 and miR-499

The exact mechanism underlying this co-regulation is the focus of future work.

Acknowledgements

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Contact: izhang@med.usyd.edu.au or nham.tran@uts.edu.au