The Oncogenic Role of miR-155

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Contents

Declaration

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List of Publications

Sam Mattiske, Rachel J Suetani, Paul M Neilsen, David F Callen (2012) The Oncogenic Role of miR-155 in Breast Cancer. *Cancer Epidemiology, Biomarkers and Prevention*. Published.

Paul M Neilsen*, Jacqueline E Noll*, Sam Mattiske*, Cameron P Bracken, Philip A Gregory, Renee B Schulz, Sue P Lim, Raman Kumar, Rachel J Suetani, Gregory J Goodall and David F Callen. (2012) Mutant p53 drives invasion in breast tumors through up-regulation of miR-155. *Oncogene*. Published

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Overview

MicroRNAs (miRs) are regulatory small noncoding RNAs that control expression of target genes by inhibiting translation and directly targeting messenger RNA (mRNA) transcripts for degradation [1]. The mature miR binds to its target by partial complementarity, usually in the 3`UTR of target mRNA. Each miR has a specific complementary seed sequence, around 7 or 8 nucleotides long. By binding to the seed sequence on the mRNA, the miR can either cause the target mRNA to be destroyed, or merely inhibit subsequent translation of the mRNA [2-4]. A single miR can regulate multiple targets [5].

miR expression profiles have been used to classify cancers, reviewed in [6], and investigations into breast cancer expression profiles have discovered abnormally high levels of particular miRs [7-10]. Studies are underway to identify the mechanisms underlying the deregulation of miRs and their association with cancer [11]. In breast cancer a small number of miRs have been found to be significantly deregulated in breast cancer tissue compared with non-malignant breast tissue [7, 9, 10, 12].

Expression profiling of miRs comparing normal breast tissue and breast tumours have found that miR-155 is upregulated in breast cancer and can act as an oncomir [9-11]. Since miRs operate by inhibiting the translation of their target mRNA, one could speculate that miR-155 targets might be critical in breast tumour progression and metastasis.

The aim of this work was to investigate the oncogenic role of miR-155 in breast cancer.

Chapter 1 is a literature review focussed on miR-155 in breast cancer, including the clinical relevance of miR-155, functional characterisation, regulation of miR-155 and target genes of miR-155. In the review, a comprehensive list of all confirmed miR-155 target genes was compiled, in order to act as a resource for future researchers investigating the functional

significance of miR-155 dysregulation. The review also encompasses the origin of this miR and subsequent processing. The main aim of the literature review was to establish the field of knowledge, in order to identify areas of interest for future research: areas involving miR-155 in breast cancer that had not been fully explored.

In Chapter 2 the upregulation of miR-155 by p63 and mutant p53 in breast cancer is investigated, as well as the novel downstream target of miR-155, ZNF652. ZNF652 was an appealing target gene to investigate, as it was found to repress drivers of invasion and metastasis and could be a key downstream target of miR-155 and thus be the basis for miR-155's oncogenic effects in breast cancer. The discovery that miR-155 was upregulated by p63 and mutant p53 was exciting, as the regulation of miR-155 is an area that has not previously been thoroughly researched (as revealed by the literature review in Chapter 1).

The regulation of miR-155 is a theme that is continued in Chapter 3, which investigates which p63 isoform is responsible for the regulation of miR-155. The scope of this work was broader and not limited to breast cancer alone, as the mechanism of miR-155 regulation could be relevant to any of the cancer types in which miR-155 is upregulated. The TAp63 and ΔNp63 isoforms have opposing effects in cancer, and understanding the mechanism of regulation of miR-155 could aid our understanding of how miR-155 becomes highly upregulated in invasive breast cancers. Furthermore, this understanding could be used to better diagnose or treat patients with invasive breast cancer.

Finally, Chapter 4 summarises the results and implications of this research.

References

1. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell. 2009; 139: 871-90.
2. Llave C, Xie Z, Kasschau KD, Carrington JC. Cleavage of Scarecrow-like mRNA targets directed by a class of Arabidopsis miRNA. Science. 2002; 297: 2053-6.
3. Rhoades MW, Reinhart BJ, Lim LP, Burge CB, Bartel B, Bartel DP. Prediction of plant microRNA targets. Cell. 2002; 110: 513-20.
4. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004; 116: 281-97.
5. Krek A, Grun D, Poy MN, Wolf R, Rosenberg L, Epstein EJ, MacMenamin P, da Piedade I, Gunsalus KC, Stoffel M, Rajewsky N. Combinatorial microRNA target predictions. Nat Genet. 2005; 37: 495-500. Epub 2005 Apr 3.
6. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. Nature. 2005; 435: 834-8.
7. Volinia S, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A. 2006; 103: 2257-61.
8. Blenkiron C, Goldstein LD, Thorne NP, Spiteri I, Chin SF, Dunning MJ, Barbosa-Morais NL, Teschendorff AE, Green AR, Ellis IO, Tavare S, Caldas C, Miska EA. MicroRNA expression profiling of human breast cancer identifies new markers of tumor subtype. Genome Biol. 2007; 8: R214.
9. Iorio MV, et al. MicroRNA gene expression deregulation in human breast cancer. Cancer Res. 2005; 65: 7065-70.
10. Hannafon BN, Sebastiani P, de Las Morenas A, Lu J, Rosenberg CL. Expression of microRNA and their gene targets are dysregulated in preinvasive breast cancer. Breast. 2011; 13: R24.
11. O'Day E, Lal A. MicroRNAs and their target gene networks in breast cancer. Breast. 2010; 12: 201. Epub 2010 Mar 19.
12. Kong W, He L, Coppola M, Guo J, Esposito NN, Coppola D, Cheng JQ. MicroRNA-155 regulates cell survival, growth, and chemosensitivity by targeting FOXO3a in breast cancer. J Biol Chem. 2010; 285: 17869-79.