In the manuscript entitled “Predictive and prognostic value of selected microRNAs in luminal breast cancer”, Amorim et al. aim to examine the prognostic and predictive capacity of miRNAs in endocrine resistant luminal breast cancer. The authors claim that "miR-30c-5p, miR-30b-5p, miR-182-5p and miR-200b-3p were found to be independent predictors of clinical benefit from endocrine therapy" and that "miR-182-5p and miR-200b-3p displayed independent prognostic value for disease recurrence in luminal BrC patients after endocrine therapy"

Strength: On the whole well written, good numbers of patients in the validation study and good to see the inclusion of normal samples for comparison  
  
Limitations: Too few samples in the discovery cohort, unlikely that the selected miRNAs reflect endocrine resistance, unclear why specific genes were selected for individual results sections e.g. 3.4, no correction for multiple testing, genes appear to be cherry-picked from discovery cohort for further analysis, use of clinical endpoints not previously defined in a breast cancer setting (ERFS)

Reviewer comments  
  
In the manuscript entitled “Predictive and prognostic value of selected microRNAs in luminal breast cancer”, Amorim et al. aim to examine the prognostic and predictive capacity of miRNAs in endocrine resistant luminal breast cancer.   
Whilst the mauscript is on the whole well written, the work suffers from a fundamental statistical power flaw at the initial miRNA discovery stage which precludes acceptance with the current sample numbers. This is discussed further below.  
  
Major comments:  
  
1. Three vs. four (Luminal A Rec vs. NRec) or four vs. four (Luminal B Rec vs. NRec) or seven vs. eight (Luminal Rec vs. NRec) is not enough samples to derive a robust gene list given the molecular heterogeneity that exists within Luminal A, B or all Luminal tumours. This initial discovery sample set is statistically underpowered to detect miRNA differences between endocrine resistant vs. non-resistant tumours and this unfortunately renders much of the analyses and validation afterwards inconsequential. With regards to heterogentity, large scale molecular studies including TCGA have demonstrated that Luminal A cancers have a large number of recurrently mutated genes at a >5% frequency including PIK3CA, CDH1, MAP3K1, GATA3, MAP2K4, FOXA1 and TP53 [1], and are mostly diploid but some show copy number changes including 1q gains and 16 q losses [1]. Related to this, Luminal A tumours have been found to be spread out across at least five different IntClust subgroups – a classification scheme mainly based on copy number changes [2]. Similarly, Luminal B tumours are cyclin D1 amplified in 56% of cases and FGFR amplified in 23% [1]. They show mutations in PIK3CA, GATA3, PTEN and TP53 and are also spread out into five different IntClust subgroups [2]. In short, this heterogenetity in gene mutation/amplification and mRNA expression levels simply cannot be controlled for when comparing only four vs four samples. This means that the miRNAs found to be differentially expressed in the discovery cohort between resistant and non-resistant tumours may not at all reflect endocrine resistance but instead underlying molecular differences between tumours. This reference, whilst microarry based, may provide some help in determining a more appropriate sample size for this comparison: [3]. Without a siginificant increase in numbers at the discovery stage this manuscript cannot be accepted.  
  
2. The authors derive a list of miRNAs that specifically try to identify differences between endocrine sensitive vs. resistant tumours. These miRNAs are further tested in a second cohort where about 20% (n = 22) of the tumours are endocrine resistant. The authors see a survival difference in uni- and multi-variable analysis when comparing the lowest quartile (P ≤ 25) of the selected miRNAs to the upper three quartiles (P > 25) (Figs 4 and 5, Tables 5 and 6). The problem here is that in Figure 2 a large overlap is seen in the distribution of these miRNAs when comparing sensitive vs. resistant tumours, particularly as the values approach zero. If you select the samples with the lowest quartile expression (P ≤ 25) for e.g. miR-30c-5p, miR-30b-5p, miR-200b-3p, how many of those approx. 34 tumours are endocrine senitive and how many are resistant? By the looks of the data in Figure 2 there will be quite a bit of overlap, which once again goes back to my point 1 above – it is very unlikely that your chosen miRNAs actually reflect endocrine resistance.   
  
  
Minor comments:  
  
1. The gene selection from the discovery cohort is very odd – why not pick e.g. the top 5 or top 10 most downregulated genes? It seems like the genes were cherry-picked?   
2. Numbers of patients in each group are missing in Figs 4 and 5, Tables 5 and 6  
3. Multivariable analyses in Table 5 should contain all variables rather than adjusting for molecular subtype, Her2 and grade separately  
4. ERFS is not a standard breast cancer clinical time to event endpoint [4, 5]  
5. What was the selection criteria for the miRNAs examined in results section 3.4?  
6. How exactly were the luminal A/B defined by ER, PR, HER2 and Ki67 – a better description need to be added. E.g. Luminal A = ER+, PR+, HER2-, Ki67 low, Luminal B = ?  
7. There are far too many comparisons being made in uni- and multi-variable analyses, nothing appears to have been adjusted for multiple testing  
  
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