Figures and figure supplements

Diagnostic potential for a serum miRNA neural network for detection of ovarian cancer

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Figure 1. Flowchart of study design. (a) Protocol for miRNA sequencing, filtering, batch adjustment and separation into the training and testing sets. (b) Protocol for model development and testing.

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Figure 2. Clinical performance characteristics of the tested models. Sensitivity (blue bars) and specificity (orange bars) of the classifiers on the testing set depending on the method of variable selection. Whiskers denote 95% Confidence Intervals. (a) – Performance of models created on the subset of miRNAs selected using the significance-based filter. (b) Performance of models created on variables selected using the CFS subset algorithm. (c) Performance of models created using variables selected by the fold change-based filter. The red arrow denotes the model with the best performance characteristics, the neural network analysis using the fold change-based filter variable.

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Figure 3. ROC curves for the neural network analysis. (a) Performance of the neural network on the training set of raw, non-batch-adjusted data (red line) and in the batch-adjusted training set (black line) (b) Performance of the neural network on raw (red line) and batch-adjusted (black line) data in the testing set.

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Figure 4. ROC curves for neural network analysis compared to CA-125. The neural network (AUC 0.93; 95% CI 0.88–0.97) significantly outperformed CA125 (AUC 0.74; 95% CI 0.65–0.83) in terms of overall operating characteristics (p=0.001).

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Figure 4---figure supplement 1. Correlations between the miRNAs (vertical axes) of the neural network and CA-125 (horizontal axes) in the cancer (red markers) and benign/borderline/control (blue markers) groups. (a) miR-23b (b) miR-29a (c) miR-32 (d) miR-320d (e) miR-1246 (f) miR-92a (g) miR-150 (h) miR-200a (i) miR305 (j) miR-1307 (k) miR-200c (l) miR-203a (m) miR-320c (n) miR-450b. None of the correlations were significant in either the training or testing set.

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Figure 4—figure supplement 2. Performance of a two-tiered algorithm for ovarian cancer diagnosis incorporating both the neural network (NN) and a CA-125 cut-off of 35 U/ml. Subjecting all negative neural network algorithm results to a second review with CA-125 would increase the probability of a false positive test result from 4.2% (5/120) to 19.2% (23/120) and a false negative rate from 5.8% (7/120) to 13.3% (16/120). If the tests were considered hierarchical so that only samples classified as negative by the neural network were then examined by CA-125, this would identify three additional cases of invasive cancer but at the expense of 19 additional false positive results. FP – false positive, TP – true positive, FN – false negative, TN – true negative.

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Figure 5. Specificity of miRNA signature for ovarian cancer compared to other diagnoses. The neural network 14 miRNA signature did not separate any other diagnoses from the control group in the published dataset by Keller, et al\(^1\). The study also included 70 healthy controls. The number of subjects \(n\) denotes the number of cases of the given diagnosis in the Keller, et al dataset. (a) Pancreatic ductal cancer \(n = 45\); (b) Prostate cancer \(n = 23\); (c) Stomach cancer \(n = 13\); (d) Other pancreatic cancers \(n = 48\); (e) Melanoma \(n = 35\); (f) Lung cancer \(n = 32\); (g) Periodontitis \(n = 18\); (h) Pancreatitis \(n = 38\); (i) Multiple sclerosis \(n = 23\); (j) Acute MI \(n = 20\); (k) Chronic obstructive pulmonary disease \(n = 24\); (l) Sarcoidosis \(n = 45\). (m) Overall, neural network was highly specific for ovarian cancer cases against all other diagnoses (i.e. healthy controls or other cancers).

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**Figure 6.** ROC curve for neural network analysis using qPCR inputs from the clinical test set.

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**Figure 7.** Change in miRNA expression from preop to post-operative day three after surgical cytoreduction. n = 27.

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Figure 8. In situ expression of selected miRNAs from the serum signature. Sections of fallopian tubes showing serous tubal intraepithelial carcinoma (STIC) lesions and Stage I high grade serous ovarian cancer (HGSOC). Lesional cells are indicated by TP53 and Ki-67 staining. (top) STIC lesion in continuity with normal fallopian tube. 20x. (middle) STIC lesion in continuity with normal fallopian tube and invasive cancer with p53-null lesion. 10x. (bottom) HGSOC intraluminal to the fallopian tube. 10x.
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Figure 9. Principal component analysis identified a prominent batch effect among the study populations. (Left) Before batch effect removal. (Right) After batch effect removal using ComBat. ERASMOS – Effects of Regional Analgesia on Serum miRNA after Oncology Surgery Study. PMP – Pelvic Mass Protocol. NECC – New England Case Control study. DOI: https://doi.org/10.7554/eLife.28932.019
Figure 10. Hierarchical clustering of the eleven statistically significant miRNAs identified using univariate analysis. While most of the patients with cancer clustered together, considerable heterogeneity was evident, and no clear separation of the groups could be achieved using any single miRNA. DOI: https://doi.org/10.7554/eLife.28932.020