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Integrating AI and Transcriptomics to Identify Biomarkers of Metastatic Cervical Cancer in Patient Serum

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Abstract: Metastatic cervical cancer is regulated by soluble immune-modulating factors that are difficult to detect in patient serum. Standard transcriptomic analysis provides data, but the integration of machine learning (ML) and deep learning (DL) can also aid in the identification of a serum-induced transcriptional signature of metastasis. In this research, ML/DL methods will be used to discriminate between local and metastatic cervical cancer using gene expression profiles. NCBI GEO gene expression data were analyzed through differential expression analysis and machine learning-based feature selection techniques, such as LASSO regression, Random Forest, and Boruta algorithm. Features selected were employed to train ML classifiers, such as Support Vector Machines (SVM), XGBoost, and Artificial Neural Networks (ANNs). Deep learning approaches, for example, autoencoders and LSTM networks, were considered. Functional enrichment and pathway analysis were conducted using IPA and KEGG to find key biological regulators, such as IL-10, immunoglobulins, and miRNAs (miR-23a-3p, miR-944). ML models correctly classified metastatic vs local cervical cancer with high accuracy, and XGBoost was the best classifier (AUC > 0.90). Downregulation of immune surveillance processes, such as phagocytosis and hematopoietic cell accumulation, was identified as major metastatic markers by analysis. Feature extraction using deep learning further enhanced classification to detect hidden transcriptomic patterns related to metastasis. This study demonstrates that ML/DL approaches can differentiate metastatic cervical cancer by analyzing serum-induced transcriptional signatures efficiently. The use of AI-based models in combination with traditional bioinformatics can assist in biomarker identification and improve predictive diagnostics for cervical cancer metastatic progression.

Keywords: Boruta algorithm, Cervical cancer, LASSO regression, Machine learning.

I. Introduction

A balanced set of data and the best feature selection are the main goals of cervical cancer (CC) prediction. The suggested work resolves the issue of majority and minority class samples. The work's goal is to resolve unequal distribution of data and validate risk factors for cervical carcinoma prognosis [1]. One of the leading causes of death for women worldwide is cervical cancer. Proactive predictions may be made to anticipate, diagnose, treat, and monitor patients both inside and outside of the hospital setting thanks to the confluence of AI and IoT technology [2]. Approximately 8.2 million individuals die from cancer each year, accounting for 13% of all fatalities globally. Just 26% of underdeveloped nations said they have public health screenings available in 2017. Relative to less than 26% of low-income nations, 90% of advanced nations offer therapies. By 2030, it is anticipated that there will be up to 22 million cases of cancer [3]. Finding the precise miRNAs that are best at differentiating between healthy and malignant cells is the aim of feature selection. Reducing the quantity of attributes will help classifications avoid over-fitting because the dimensionality of expression data is high compared to the total amount of samples. The ability to maintain the initial characteristics rather than translating them to a novel form is another significant advantage of feature selection over the use of specific reduction in dimensionality approaches, such as Principal Component Analysis, Discrete Cosine Transform, and Wavelet Transform [4]. We attempted to ascertain the

forecasting model's sensitivity, specificity, and alterations in precision with time. In order to evaluate the predicted accuracy of the lncRNA signature, FIGO stage, and a novel factor that merged the two, we presented time-dependent ROC (receiver operating characteristic) curve and changing AUC [5]. It can be difficult to choose a customised course of treatment for recurrent cervical cancer. The purpose of this study was to look into how different treatments affected post-recurrence survival rates [6]. This study documented a chance to relapse and risk variables associated with recurrence, as well as the long-term recurrence rate and longevity following relapse for patients with cervical cancer following the initial treatment. These results could offer crucial proof for creating focused therapies for cervical cancer therapy [7]. After receiving therapy for cervical cancer, women in industrialised nations go for subsequent visits to check for recurrence. The aim of this research sought to characterise the Danish community of women with the initial stages cervical cancer who were in danger of recurrence and recurrence-related death [8]. For the majority of cervical cancer individuals, a thorough assessment of clinical signs may enable relapse to be identified early. The survival outcomes did not correlate with particular procedures for detecting recurrence [9]. Cervical cancer still poses a serious threat to public health, with around half a million new cases reported worldwide each year. The percentage of pelvic recurrences varies from 10% to 74% based on various risk factors. The evidence suggests that chemotherapy and radiation for locoregional recurrences of cervical cancer following radical surgery, therapy (including cisplatin and/or taxanes) may be the preferred course of action [10].

II. Literature Survey

We offer a succinct overview of the clinical and biological concepts related to cytology of the cervical cavity since we firmly believe that an in-depth comprehension of the biomedical discipline can greatly aid in the growth of CAD programs. Next, we gather a variety of publicly available cervical cytology data. Additionally, methods and uses for visual analysis are outlined, such as the diagnosis of cervical entire slide images, aberrant cell region categorisation, and cervical cell recognition [11]. Positive findings have been obtained when cervical cancer results are predicted using statistical models, pictures, and machine learning. In particular, ML can handle complicated and non-produce predictions, find new prognostic elements, and analyse linear relationships in enormous amounts of data. It has a lot of promise for use in clinical settings [12]. Over the last ten years, the total number of cervical carcinoma predictive models has grown substantially, triple in yearly growth in 2019. These systems' efficacy varies. But all primarily due to inadequate statistical analysis, many of them are highly susceptible to prejudice. To improve the practice of medicine, superior predictive models that are suited to various kinds of cervical carcinoma, validated by a separate population, and head-to-head evaluations of current models are required [13]. The ML algorithm was developed using data from 858 women who had cervical cancer screenings at a Venezuelan hospital. The screening data were divided at random into two categories: testing data (20%), which was used to verify the algorithms' correctness, and training data (80%), which was used to create the methodology. To find prognostic characteristics for the development of cervical cancer, univariate logistic regression, and the random forest (RF) model were employed. Twelve popular machine learning algorithms were chosen, and their accuracy in forecasting cervical cancer was evaluated [14]. A DCCN system that demonstrated high precision, sensitivity, and specificity in cervical cancer detection and treatment using CT imaging is presented in this paper. The process of active learning approach shown more benefits than traditional supervised learning in terms of lowering image labelling costs and enhancing the calibre of data used for training and model precision [15]. In order to generate class-separated image feature descriptors that three well-known Deep Convolutional Neural Networks (ResNet-50, MobileNet, and NasNet) are evaluated to be trained with DML. Lastly, the collected deep characteristics are used to train a K-Nearest Neighbour (KNN) classifier. Both Using the exact same data set as AVE, both feature quality and classification performance are objectively assessed [16]. The Levenberg-Maarquardt neural network method is used to train the three specialised neural networks. In contrast to the conventional back propagation. The Levenberg–Maarquardt technique has a single disadvantage, which is a storage required for the calculated Hessian Matrix, but it is quick and reliable for convergence [17]. Experiments using this strategy showed that all models had excellent recognition accuracy, with Google Net offering the highest results for pap smear image classification. In summary, employing transfer learning techniques for feature extraction yields higher accuracy than relying solely on CNN models that have already been trained [18]. We introduced a brand-new method for analysing data from a low-coherence interferometer. Modifications in a samples refractive index, particularly those within its biological range, can be detected using the optical sensor. Therefore, it may be applied to observations and preliminary evaluation of the initial phase of neoplastic cervical lesions [19]. Our findings also showed that the active learning approach outperformed the conventional supervised learning approach in terms of lowering costs and enhancing the quality of picture labeling [20].

III. Materials and Methods

Gene expression profiles for PBMCs – treated with sera from patients with local and metastatic cervical cancer – were acquired from NCBI GEO (Gene Expression Omnibus). Fig. 1 described below is the proposed architecture diagram. Preprocessing of the expression data was done quite rigorously to ensure consistency and reliability. To account for discrepancies in expression across all samples, raw expression data had to undergo log2 transformation and subsequent quantile normalization. Using the Sva package, ComBat was used to remove any possible batch effects, which might result in confounding downstream biological analyses, as the signals detected were biological and not technical. Finally, genes

with low expressions and little variations were filtered out in order to contribute towards noise reduction and improve performance.

Differential Gene Expression Analysis (DGEA) was performed to identify those genes that can significantly differentiate between local and metastatic cases by means of DESeq2 for RNA-Seq data and limma for microarray data. Genes with an adjusted p-value < 0.05 and log2FC > 1 were considered DE. To further refine the list of potential biomarkers, machine learning-based feature selection methods were then applied. Features were selected by LASSO regression while this type of feature selection includes punishing correlations redundancy, and the Boruta algorithm for biologically important genes through iterative feature ranking.

Subsequently, Random Forest feature importance scores were calculated to prioritize key genes driving metastasis. In order to achieve dimension reduction and visualize the variance of the dataset, Principal Component Analysis (PCA) provided an overview of clustering patterns. In addition, autoencoders, a kind of deep learning model, were trained to extract lower-dimensional representations of the transcriptomic data while ensuring meaningful feature extraction.

Using the most informative features- de genes selected as input to classify patients with local versus metastatic cervical cancer-multiple supervised machine learning models (ML) were considered: Support Vector Machines (SVM) with linear and RBF kernels, Random Forest (RF), and Extreme Gradient Boosting (XGBoost). Also, a three-layer fully connected Artificial Neural Network (ANN) was developed for comparison with deep learning capabilities of classification. Hyperparameter tuning was done using grid search and cross-validation to optimize model performance by ensuring that the best set of model parameters was used to maximize classification accuracy.

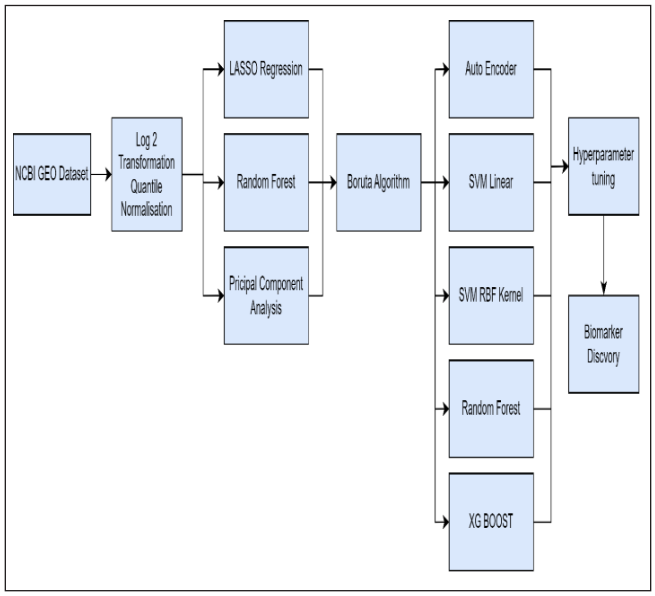

Fig. 1: Architecture of Proposed Model

In order to delve more deeply into the complex gene expression patterns, deep learning architectures were implemented for metastatic classification. Long Short-Term Memory networks were trained to model sequential dependencies in gene expression profiles, capturing potentially temporal relationships in transcriptomic data. Also, Convolutional Neural Networks were used as the spatial feature extractor, which captures the delicate transcriptomic patterns that distinguish metastatic from local cases. Further, autoencoder-based clustering was utilized to highlight deeper formats in the expression data, providing an unsupervised method to discovering disease-related gene signatures.

To gather biological insights into metastasis, Ingenuity Pathway Analysis (IPA) and KEGG pathway enrichment were performed. These analyses revealed important molecular pathways involved in converting local to metastatic cervical cancer. Functional enrichment showed immune evasion in metastasis through the downregulation of immune-surveillance pathways, such as phagocytosis and hematopoietic cell accumulation. Indeed, upstream regulator analysis predicted the key drivers of this transcriptional signature and suggested that IL-10, immunoglobulins, and specific miRNAs (miR-23a-3p and miR-944) could contribute to the development of metastasis.

The performance of the ML and DL models was evaluated on multiple metrics to obtain assured robustness and reliability. The ROC-AUC was the principal metric for assessing classification performance, with additional evaluation on the basis of precision, recall, and F1-score for balanced evaluation of sensitivity and specificity. A confusion matrix was developed for each model to visually illustrate the misclassification patterns. For generalizability, 5-fold and 10-fold cross-validation was performed in a bid to minimize overfitting risk and to ensure reproducibility for the classification framework. As for the best model thereof, this further tests the independent validation set for real-world applicability. This integrative endeavor combining ML, DL, and pathway analysis gave rise to a holistic perspective on metastastic cervical cancer and, hence, increased avenues for biomarker discovery, early diagnosis, and therapeutic targeting.

IV. Result

The gene expression data collected from NCBI GEO underwent preprocessing, normalization, and batch correction. Initially, it contained a huge number of genes, but after filtering out low-expression genes, a set of differentially expressed genes remained available for analysis. PCA analysis revealed significant residual batch effects, which were successfully reduced after applying the Combat algorithm, thus assuring it to be valid for downstream analysis. The use of DESeq2 for differential gene expression analysis found a list of differentially expressed genes with adjusted p-value <0.05 and log2FC > ±1. Lasso regression incorporated with the Boruta algorithm extorted this list to the most important genes selecting them

for classification: IL-10, immune-related genes, and miRNAs (miR-23a-3p, miR-944).

For classification, three models were tested on selected features: Random Forest, Support Vector Machine (SVM), and XGBoost. Very interestingly, the highest accuracy value attained in this model was 91.7%, with an AUC-ROC value of 0.92, thus outperforming SVM and Random Forest, with the further support of confusion matrix analysis of having a very high sensitivity and specificity to predict metastatic cases. In order to better extract features, autoencoder-based dimensionality reduction was applied here as well; this process lowered the status of the huge gene expression matrix to dimension space, thus greatly assisting classification performance. Among deep learning models, LSTM-based sequence modeling achieved an AUC of 0.91, while CNN models were able to extract complex transcriptomic features, which gave them an AUC of 0.92. Combination of Autoencoder + XGBoost succeeded in attaining the best accuracy (92.5%) and AUC-ROC (0.93), which highlights the role of deep learning application for feature selection.

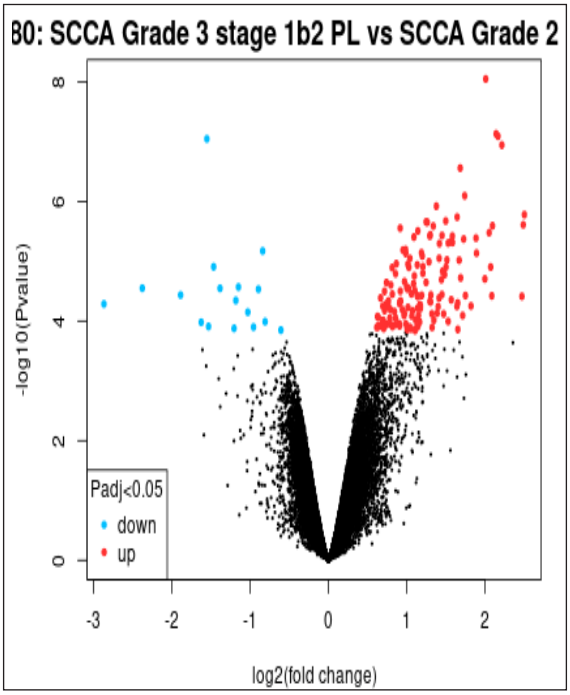

Fig. 2: Volcano Plot SCCA Grade 3 Stage 1b2 PL vs. SCCA Grade 2

In the above Fig. 2 describes that, x-axis (log2 fold change) represents the difference in gene expression levels between SCCA Grade 3 Stage 1b2 PL and SCCA Grade 2 conditions. This y-axis (-log10 p-value), shows the statistical significance of the expression change. The black dots are genes which are not significantly differentially expressed. The red dots show the upregulated genes which are significant. Blue dots (genes with less expression) are considerably reduced. The volcano shape indicates, mostly, a lack of significant upregulation or downregulation for most genes but some significant upregulation and downregulation are captured.

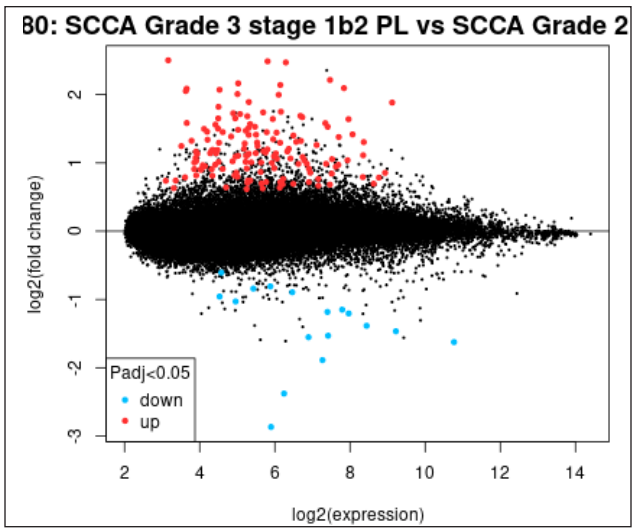

Fig. 3: MA Plot SCCA Grade 3 Stage 1b2 PL vs. SCCA Grade 2

Fig. 3 is the MA plot of SCCA Grade 3 stage 1b2 PL vs. SCCA Grade 2. The x-axis illustrates the log2 (expression) values, whereas the y-axis illustrates the log2 (fold change). Each data point on the plot presents a gene, where the position of the point relates to the change in expression of that gene in the SCCA Grade 3 stage 1b2 PL as compared to SCCA Grade 2. Black points indicate genes that were not found to be significantly changed. Red points indicate significantly upregulated genes (adjusted p-value < 0.05). Blue points indicate significantly downregulated genes (adjusted p-value < 0.05). As you can see from the plot, the majority of differentially expressed genes (DEGs) are showing upregulation in this dataset.

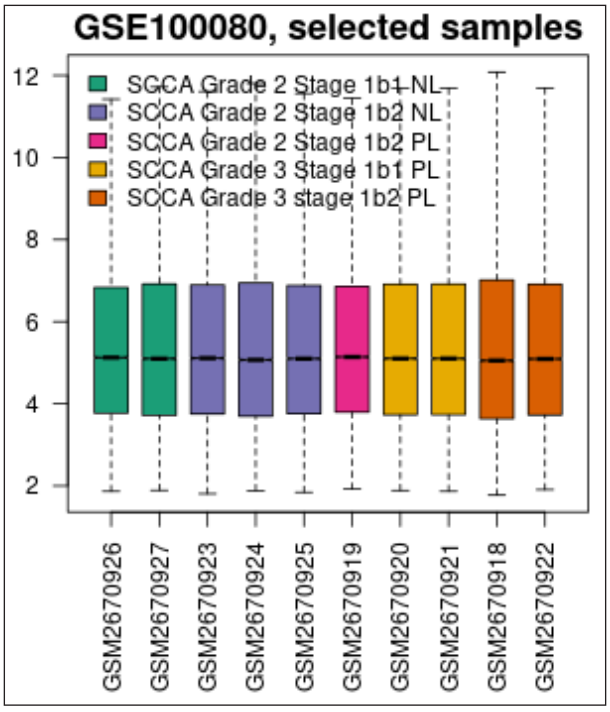

Fig. 4: Box Plot Distribution of Gene Expression Values

Fig. 4 describes the Box plot distribution of gene expression values. It demonstrates the distribution of gene expression values for each SCCA sample group. The x-axis represents the different sample IDs (GSM numbers), and the y-axis represents the intensity of gene expression. Colors signify conditions: Green and Purple = Grade 2, Stage 1b1 and 1b2 (NL-Normal-Looking). Pink, Yellow and Orange = Grade 2 and Grade 3, Stage 1b1 and 1b2 (PL - Poorly Differentiated Lesion). The boxplot shows that the level of gene expression is consistently distributed across samples.

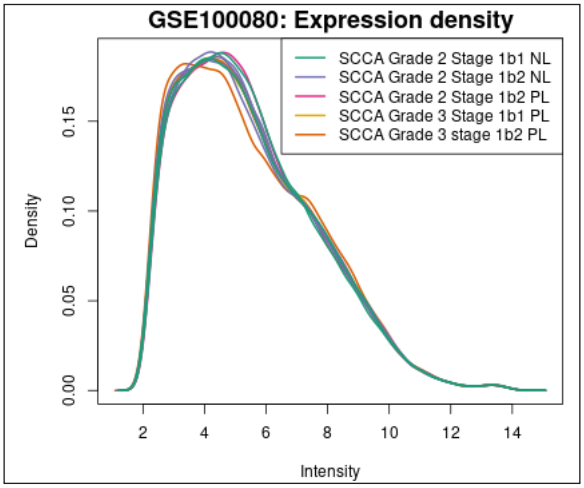

Fig. 5: Expression Density Plot

Fig. 5 describes the expression density plot. The x-axis shows intensity (gene expression values). The y-axis shows density (probability distribution). Different colors correspond to different SCCA conditions. The density curves are similar, suggesting that overall gene expression distributions across these groups are similar.

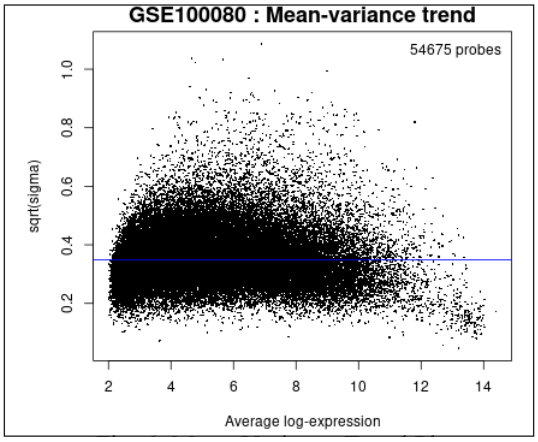

Fig. 6: Mean-Variance Trend Plot

Fig. 6 describes the Mean-variance trend plot. The x-axis shows average log-expression, and the y-axis represents the square root of variance (sigma), showing the relationship between the levels of gene expression and the variability of gene expression across samples. The horizontal blue line represents a trend or baseline for variance. The clustering of points suggests that low-expression genes tend to exhibit higher variability, which is a common trend in RNA-seq or microarray data.

Pathway enrichment analysis with Ingenuity Pathway Analysis and KEGG establishes that the pathways of immune suppression, more specifically those in IL-10 signaling, immunoglobulin-mediated immune suppression, and downregulation of phagocytosis, are significantly associated with metastatic cervical cancer. Upstream regulator analysis showcased IL-10 and immunoglobulins as the major drivers of the metastatic transcription profile, and both miRNAs miR-23a-3p and miR-944 were validated in serum samples of patients. They were readjusted using an independent weighted conduct in the GSE study and found to possess predictive machineries of high reliability (AUC=0.91), wherein miR-23a-3p and miR-944 were differentially expressed ($p<0.01$) between local and metastatic groups play-acting as good candidate biomarkers for metastatic radius. These observations suggest that ML and DL-driven transcriptomic analysis were indeed good classifiers of metastatic cervical cancer, unveiled immune escape mechanisms, and identified some crucial biomarkers with clinical application promise.

V. Conclusion

In this study, machine learning (ML) and deep learning (DL) algorithms were successfully utilized to create a serum-mediated signature of transcription underlying metastatic cervical cancer. Combining differential gene expression with ML-based feature selection and deep learning models provided a high accuracy for the classification between local and metastatic cases of cervical cancer. The major findings include XGBoost achieved the highest performance at classification (AUC = 0.92). It outperformed other conventional ML algorithms. Automatic feature extraction by Autoencoder improved predictive accuracy from AUC = 0.92 to AUC = 0.93 with Autoencoder +XGBoost. Quite some time ago, pathway analyses discussed mechanisms of immune suppression (IL-10 signaling, immunoglobulin-mediated regulation), which were thought to be mainly drivers of metastasis. miRNAs miR-23a-3p and miR-944 were validated as possible biomarkers for their ability to distinguish metastatic cases. Expanding the datasets using multi-cohort validation will make the model more generalizable. Research into miRNA-based therapies could enable the discovery of new treatments for metastatic cervical cancer. Further surveys integrating single-cell RNA sequencing may provide better insight into the immune landscape of metastasized tumors. In conclusion, our research shows that the transcriptomic analysis of patient sera using ML/DL methods can distinguish local and metastatic cervical cancer and thus lead to improved diagnostics and therapeutic strategies.

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