PROBLEM SOLVING PROTOCOL

XOmivAE: an interpretable deep learning model for cancer classification using high-dimensional omics data

Eloise Withnell,1,2,† Xiaoyu Zhang,1,*,† Kai Sun1 and Yike Guo1,3

1Data Science Institute, Imperial College London, SW7 2AZ, London, UK, 2Department of Health Informatics, University College London, WC1E 6BT, London, UK and 3Department of Computer Science, Hong Kong Baptist University, Hong Kong, China

*Corresponding author. x.zhangu18@imperial.ac.uk†The first two authors contributed equally to this paper.

Abstract

The lack of explainability is one of the most prominent disadvantages of deep learning applications in omics. This “black box” problem can undermine the credibility and limit the practical implementation of biomedical deep learning models. Here we present XOmivAE, a variational autoencoder (VAE) based interpretable deep learning model for cancer classification using high-dimensional omics data. XOmivAE is capable of revealing the contribution of each gene and latent dimension for each classification prediction, and the correlation between each gene and each latent dimension. It is also demonstrated that XOmivAE can explain not only the supervised classification but the unsupervised clustering results from the deep learning network. To the best of our knowledge, XOmivAE is one of the first activation level-based interpretable deep learning models explaining novel clusters generated by VAE. The explainable results generated by XOmivAE were validated by both the performance of downstream tasks and the biomedical knowledge. In our experiments, XOmivAE explanations of deep learning based cancer classification and clustering aligned with current domain knowledge including biological annotation and academic literature, which shows great potential for novel biomedical knowledge discovery from deep learning models.

Key words: explainable artificial intelligence, deep learning, cancer classification, omics data, gene expression

Introduction

High-dimensional omics data (e.g., gene expression and DNA methylation) comprises up to hundreds of thousands of molecular features (e.g., gene and CpG site) for each sample. As the number of features is normally considerably larger than the number of samples for omics datasets, genome-wide omics data analysis suffers from the “curse of dimensionality”, which often leads to overfitting and impedes wider application. Therefore, performing feature selection and dimensionality reduction prior to the downstream analysis has become a common practice in omics data modelling and analysis [Meng et al., 2016]. Standard dimensionality reduction methods like Principal Component Analysis (PCA) [Ringnér, 2008] learn a linear transformation of the high-dimensional data, which struggles with the complicated non-linear patterns that are intractable to capture from omics data. Other non-linear methods such as t-distributed Stochastic Neighbor Embedding (t-SNE) [van der Maaten and Hinton, 2008] and Uniform Manifold Approximation and Projection (UMAP) [McInnes et al., 2020] have become increasingly popular, but still have limitations in terms of scalability.

Deep learning has proven to be a powerful methodology for capturing non-linear patterns from high-dimensional data [LeCun et al., 2015]. Variational Autoencoder (VAE) [Kingma and Welling, 2014] is one of the emerging deep learning methods that have shown promise in embedding omics data to lower dimensional latent space. With a classification downstream network, the VAE-based model is able to classify tumour samples and outperform other machine learning and deep learning methods [Zhang et al., 2019, Azarkhalili et al., 2019, Hira et al., 2021, Zhang et al., 2021]. Among them, OmiVAE [Zhang et al., 2019] is one of the first VAE-based multi-omics deep learning models for dimensionality reduction and tumour type classification. An accuracy of 97.49% was achieved for the classification of 33 pan-cancer tumour types and the normal control using gene expression and DNA methylation profiles from the Genomic Data Commons (GDC) dataset [Grossman et al., 2016]. Similar
to OmiVAE, DeePathology [Azarkhalili et al., 2019] applied two types of deep autoencoders, Contractive Autoencoder (CAE) and Variational Autoencoder (VAE), with only the gene expression data from the GDC dataset, and reached accuracy of 95.2% for the same tumour type classification task. Hira et al. [2021] adopted the architecture of OmiVAE with Maximum Mean Discrepancy VAE (MMD-VAE), and classified the molecular subtypes of ovarian cancer with an accuracy of 93.2-95.5%. Zhang et al. [2021] synthesised previous models and developed a unified multi-task multi-omics deep learning framework named OmiEmbed, which supported dimensionality reduction, multi-omics integration, tumour type classification, phenotypic feature reconstruction and survival prediction. Despite the breakthrough of aforementioned work, a key limitation is prevalent among deep learning based omics analysis methods. Most of these models are “black boxes” with lack of explainability, as the contribution of each input feature and latent dimension towards the downstream prediction is obscured.

Various strategies have been proposed for interpreting deep learning models. Among them, the probing strategy, which inspects the structure and parameters learnt by a trained model, have been shown to be the most promising [Azodi et al., 2020]. Probing strategies generally fall into one of three categories: connection weights-based, gradient-based and activation level-based approaches [Montavon et al., 2018]. The connection weight-based approach sums the learnt weights between each input dimension and the output layer to quantify the contribution score of each feature [Garson, 1991, Olden and Jackson, 2002]. Way and Greene [2018] and Bica et al. [2020] adopted this probing strategy to explain the latent space of VAE on gene expression data. However, the connection weight-based approach can be limited or even misleading when positive and negative weights offset each other, when features do not have the same scale, or when neurons with large weights are not activated [Shrikumar et al., 2017]. In the gradient-based approach, contribution scores (or saliency) are measured by calculating the gradient when the input are perturbed [Simonyan et al., 2014]. Dincer et al. [2018] applied a gradient-based approach, Integrated Gradients [Sundararajan et al., 2017], to explain a VAE model for gene expression profiles. This approach overcomes limitations of the connection weights-based method. Despite this, it is inaccurate when small changes of the input do not affect the output [Shrikumar et al., 2017]. The activation level-based approach conquers these drawbacks by comparing the feature activation level of an instance of interest and a reference instance [Azodi et al., 2020]. An activation level-based based method named Layer-wise Relevance Propagation (LRP) has been used to explain a deep neural network for gene expression [Hanczar et al., 2020]. Nevertheless, LRP can produce incorrect results with model saturation [Shrikumar et al., 2017]. Deep SHAP [Lundberg and Lee, 2017], which applies the key principles from DeepLIFT [Shrikumar et al., 2017], has been used in a variety of biological applications [Tasaki et al., 2020, Lemsara et al., 2020]. However, there is a lack of research on the application of Deep SHAP to interpret the latent space of VAE models and the VAE-based cancer classification using gene expression profiles.

Here we proposed explainable OmiVAE (XOmiVAE), a VAE-based explainable deep learning omics data analysis model for low dimensional latent space extraction and cancer classification. OmiVAE took advantage of Deep SHAP [Lundberg and Lee, 2017] to provide the contribution score of each input molecular feature and omics latent dimension for the cancer classification prediction. Deep SHAP was selected as the interpretation approach of XOmiVAE due to its ability to provide more accurate explanations over other methods, which likely provides better signal-to-noise ratio in the top genes selected. With XOmiVAE, we are able to reveal the contribution of each gene towards the prediction of each tumour type using gene expression profiles. XOmiVAE can also explain unsupervised tumour type clusters produced by the VAE embedding part of the deep neural network. Additionally, we raised crucial issues to consider when interpreting deep learning models for tumour classification using the probing strategy. For instance, we demonstrate the importance of choosing reference samples that makes biological sense and the limitations of the connection weight-based approach to explain latent dimensions of VAE. The results generated by XOmiVAE were fully validated by both biomedical knowledge and the performance of downstream tasks for each tumour type. XOmiVAE explanations of deep learning based cancer classification and clustering aligned with current domain knowledge including biological annotation and literature, which shows great potential for novel biomedical knowledge discovery from deep learning models.

Methods

Datasets and pre-processing

The Cancer Genome Atlas Program (TCGA) [Network et al., 2013] pan-cancer dataset, which comprise gene expression profiles of 33 various tumour types, was used in the experiment as a example to demonstrate the explainability of XOmiVAE. A total of 9,081 samples from TCGA were selected for training and testing our proposed model, of which 407 were normal tissue samples. The TCGA dataset was downloaded from UCSC Xena data portal [Goldman et al., 2020] (https://xenabrowser.net/datapages/, accessed on 1 May 2019). We followed the same omics data pre-processing step as OmiVAE [Zhang et al., 2019] and OmiEmbed [Zhang et al., 2021]. Genes targeting the Y chromosome, genes with zero expression level in all samples, and genes with missing values (N/A) in more than 10% of the samples were removed to ensure the gene expression data was fair and clean across samples. Furthermore, the remaining N/A values that did not reach the 10% threshold were replaced by the expression level of corresponding genes. The Fragments Per Kilobase of transcript per Million mapped reads (FPKM) values were normalised to the unit interval of 0 to 1 to the meet input requirement of the network. The phenotype data of each sample was also downloaded from UCSC Xena, which is comprised of age and gender of the subjects and primary site and disease stage of the samples. The the detailed cancer subtype information of each tumour sample was obtained from Sanchez-Vega et al. [2018].

Explainable OmiVAE (XOmiVAE)

Based on vanilla OmiVAE, we proposed an interpretable deep learning model for cancer classification using high-dimensional omics data, named Explainable OmiVAE, aka XOmiVAE. The overall architecture of XOmiVAE was illustrated in Figure 1. The input omics data, which was genome-wide gene expression profiles here, was first passed through a VAE embedding network to reduce the dimensionality of the input data from 58,043 to 128. The encoder of the embedding network contained
two output vector, the mean vector $\mu$ and the standard deviation vector $\sigma$, which defined the Gaussian distribution $\mathcal{N}(\mu, \sigma)$ of the latent variable $z$ given the input omics data $x$. In order to enable backpropagation for the sampling step, the reparameterisation trick was applied according to Equation 1:

$$z = \mu + \sigma \epsilon$$  \hspace{1cm} (1)$$

where $\epsilon$ is a random variable sampled from a unit Gaussian distribution $\mathcal{N}(0, 1)$. The VAE network of XOmiVAE was optimised by maximising the variational Evidence Lower BOund (ELBO) defined in Equation 2:

$$\text{ELBO} = E_{q_{\phi}(z|x)} \left[ - \log q_{\phi}(z|x) + \log p_{\theta}(x, z) \right].$$  \hspace{1cm} (2)$$

$q_{\phi}(z|x)$ is the variational distribution introduced to approximate the true posterior distribution $p_{\theta}(z|x)$, where $\phi$ is the set of learnable parameters of the encoder and $\theta$ is the set of learnable parameters of the decoder. Equation 2 can further transform to Equation 3:

$$\text{ELBO} = E_{q_{\phi}(z|x)} \log p_{\theta}(x|z) - D_{\text{KL}}(q_{\phi}(z|x) \parallel p_{\theta}(z))$$  \hspace{1cm} (3)$$

where $p_{\theta}(x|z)$ is the conditional distribution, and $D_{\text{KL}}$ is the Kullback–Leibler (KL) divergence between two probability distributions.

A three-layer classification neural network was attached to the bottleneck layer of the VAE deep embedding network for the tumour type classification downstream task. The latent vector $\mu$ was fed to the classification network as the input and passed through two hidden layers with 128 neurons and 64 neurons respectively before the probability of each tumour type was obtained by the softmax activation function in the output layer.
We defined the loss function of the classification network as the cross-entropy between the ground-truth tumour type \( y \) and predicted tumour type \( y' \), as shown in Equation 4:

\[
L_{\text{class}} = CE(y, y')
\]

Thus, the overall loss function of the whole model was a weighted combination of the VAE loss \( L_{\text{VAE}} \) and the classification loss \( L_{\text{class}} \), which was defined in Equation 5:

\[
L_{\text{total}} = \alpha L_{\text{VAE}} + \beta L_{\text{class}}
\]

\( \alpha \) and \( \beta \) weighted the two losses during training. The hyper-parameters used to train this model was listed in Table 1.

XOmivae has the ability to explain both the supervised tumour type classification results, which was illustrated in Figure 1 (A), and the unsupervised omics data clustering results, which was illustrated in Figure 1 (B). Based on the vanilla OmiVAE, we integrated the Deep SHAP explanation approach to XOmivAE in a customised way. Deep SHAP inherited the key principle from DeepLIFT, which is the “summation-to-delta” property. This property means that the sum of the contributions over the input equals the difference-from-reference of the output [Shrikumar et al., 2017], which can be formalised by Equation 6:

\[
\sum_{i=1}^{n} C_{\Delta x_i, \Delta o} = \Delta o
\]

where \( \Delta o \) is the difference between the output of the reference sample and the output of the target sample, which is \( \Delta o = f(x) - f(r) \). \( x \) is the target gene expression profile, \( r \) is the reference gene expression profile, \( \Delta x_i = x_i - r_i \), \( i \) is the gene index and \( n \) is the number of genes used in the experiment [Lundberg and Lee, 2017]. Another representation of the “summation-to-delta” property was demonstrated in Figure 1 (C). This property enables the calculation of the Shapley values, which indicate how to allocate contribution of each prediction result among the input features. Larger Shapley values, therefore, represent more important genes for the prediction of certain tumour type.

As for the implementation, a trained network was first passed to the Deep SHAP explainer object of XOmivAE alongside the reference values to calculate the SHAP values from. The computation graph of the model was then able to effectively guide the explainer through the network to calculate the activation of neurons according to principles used by DeepLIFT. The original Deep SHAP was also modified to ensure it could take either the latent vector or the classification output vector as the output values for the contribution analysis. As recommended by Shrikumar et al. [2017], we used the pre-activation output instead of the post-softmax probabilities to calculate feature contribution scores. For each prediction, \( n \) SHAP values corresponding to \( n \) genes or \( n \) latent dimensions were calculated to determine the contribution. The absolute values of SHAP values for each feature were averaged over a group of samples with the same label to indicate the overall contribution for each feature, as shown in Figure 1 (C). This avoids the issue of positive and negative SHAP values offsetting each other when they were averaged across samples. To reveal the contribution of each omics latent dimension in unsupervised tasks, we calculated the mean and standard deviation of the latent vector values (\( \mu \) values) for the two groups of samples and applied a Welch’s t-test to obtain the most statistically significant dimension that separates the two groups. The correlation between the each latent variable and each gene was obtained by backpropagating the latent vector through the Deep SHAP explainer object of XOmivAE.

Bioinformatics analysis

To evaluate the contribution results obtained by XOmivAE, we compared genes with high contribution scores with the differentially expressed genes (DEGs) between normal and cancer samples for each tumour type. We used an R Bioconductor package TCGAbiolinks [Colaprico et al., 2015] to conduct the differential gene expression analysis. The DEGs were selected according to the cut-off of 0.05 for the False Discovery Rate (FDR) adjusted p-value and the threshold of 3 for the absolute log2 fold change. To reveal the biological implication of the top genes with high contribution scores, we used the Broad Institute’s Gene Set Enrichment Analysis (GSEA) software to perform pathway enrichment analysis [Subramanian et al., 2005]. Additionally, we used the curated gene sets from online databases including the Gene Ontology (GO) [Consortium, 2004], Kyoto Encyclopedia of Genes and Genomes (KEGG) [Kanehisa and Goto, 2000], and the Reactome pathway database (Reactome) [Fabregat et al., 2018] to test subtypes pathways. g:Profiler [Raudvere et al., 2019] was used to obtain and visualise the top pathways. GeneCard [Stelzer et al., 2016] was used to obtain the specific gene set for each TCGA tumour type.

Results and discussion

Multi-level explanation of XOmivAE

Most important genes for cancer classification

We trained XOmivAE on the TCGA pan-cancer dataset and calculated the contribution score of each gene for the prediction of each tumour type. The model achieved high accuracy for differentiating between normal and tumour tissue. For instance, the classification accuracy of BRCA invasive Carcinoma (BRCA) and normal breast tissue was 99.6% and 100% respectively. The contribution scores followed a power-law distribution, which suggested the majority of input features (i.e., genes) were unimportant for cancer prediction (see Supplementary Figure S1–S2). As an example, we illustrated the top 10 genes with the highest scores that contributed most to the BRCA prediction in Figure 2. This demonstrated the explainability of XOmivAE by revealing the contribution of each input feature (i.e., gene). To validate whether the top genes found by XOmivAE made biological sense, we analysed the biological function of the most and least important genes. The top genes are known to be related to BRCA. For example, the top 1 gene for BRCA, SCGB2A2, which codes for the protein Mammaglobin A, is highly specific of breast tissue and
increasingly being used as a marker for breast cancer [Lacroix, 2006]. The second most important gene, AZGP1, is associated with an aggressive breast cancer phenotype [Parris et al., 2014]. On the contrary, the 20 least important genes are either non-coding RNAs or pseudogenes with minor biological function, which are reasonable to be irrelevant when distinguishing breast tumour from normal breast tissue. A list of top genes with their contribution scores for the other 32 tumour types was also obtained by XOmiVAE and shown in Supplementary Figure S3 to S6.

**Most important dimensions for cancer classification**

By passing an interim layer to the Deep SHAP explainer object of XOmiVAE, it is possible to obtain the most important neuron for a prediction in a specific layer. In the case of OmiVAE, the most intriguing interim layer to explain is the bottleneck layer, where the high dimensional gene expression data is reduced into a latent representation with lower dimensionality, 128 dimensions in our scenario. Therefore, the input of the first layer in the classification network was intercepted and explained using XOmiVAE. As an example, we show the top dimensions for different subtypes of kidney tumours: kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC) and kidney renal papillary cell carcinoma (KIRP). The top two dimensions are different among kidney tumour subtypes and the third one was shared (Table 2), which is therefore possible the dimension responsible for separating the kidney located tumours. Additionally, it is practicable to find the most associated genes and, therefore, the most related biological pathways to a specific dimension. We investigate the top 15 genes for the shared kidney dimension 35 as an example (see Supplementary Figure S12). These results can be obtained for every dimension and every tumour type.

**Validation by biomedical knowledge**

**Biomedical meaning of the top genes**

To validate the top genes revealed by XOmiVAE, we first compared the genes, as ranked by contribution, for each tumour type, with genes associated with the corresponding tumour type from GeneCard [Stelzer et al., 2016]. GeneCard was chosen due to its comprehensive disease gene sets, which are integrated from around 150 different web sources, and therefore covered the majority of tumour types in our analysis. We selected the genes to compare at 100 different thresholds, spaced evenly from 1 (the most important gene) to 58,043 (the total number of genes). XOmiVAE were compared to different thresholds of a random sample of genes (averaged over 10 random seeds) and state-of-the-art methods, including Saliency [Simonyan et al., 2014], Input X Gradient (an extension of Saliency) and GradientSHAP [Lundberg and Lee, 2017]. The results were plotted as a ROC curve for the True Positive Rates (TPR) and False Positive Rates (FPR). The TPR were calculated by,

\[
\frac{\text{# of top genes which are GeneCard disease genes}}{\text{# of GeneCard disease Genes}}.
\]

And the FPR were calculated by,

\[
\frac{\text{# of top genes which are not GeneCard disease genes}}{\text{# of genes not associated with the GeneCards disease}}.
\]

21 tumour types had gene sets found in GeneCard and were therefore chosen for analysis. The ROC curves and AUC metrics are shown in Supplementary Figures S7 to S9. Two example ROC curves are illustrated in Figure 3. All 33 tumour types had an AUC metric considerably higher than the random samples which suggests that the most important genes returned by XOmiVAE are biologically relevant. The average AUC metric among all 33 tumour types of XOmiVAE and three state-of-the-art methods was listed in Table 3. XOmiVAE outperformed all of the three state-of-the-art methods. To further explore and understand the top genes revealed by XOmiVAE, they were evaluated using gene set enrichment analysis. We used gProfiler [Raudvere et al., 2019], a web server for functional enrichment analysis, to identify the most significant GO terms enriched in the top genes for each tumour type. Supplementary Table S1 lists the GO terms that are significantly overrepresented in top BRCA genes. The most significant GO terms related to the extracellular matrix organisation, an area of focus within breast cancer research.

### Table 2. The top dimensions for kidney tumour subtypes: KICH, KIRC and KIRP.

| Dimension rank | KICH | KIRC | KIRP |
|----------------|------|------|------|
| 1st            | 45   | 20   | 42   |
| 2nd            | 50   | 83   | 67   |
| 3rd            | 35   | 35   | 35   |
| 4th            | 111  | 53   | 125  |
| 5th            | 42   | 103  | 45   |

### Table 3. The average AUC score across all 33 tumour types for the ranked gene importance scores compared to the GeneCard genes.

| Methods          | Average AUC | Standard deviation |
|------------------|-------------|--------------------|
| Saliency         | 0.5331      | 0.0839             |
| GradientSHAP     | 0.7682      | 0.0578             |
| Input X Gradient | 0.7762      | 0.0767             |
| XOmiVAE          | **0.7950**  | 0.0673             |
To further understand the latent dimensions used for LUAD classification, we tested whether there was a dimension that contrasts with the most important pathways that these dimensions used for LUAD classification. XOmivAE is able to capture this as it detects the activation of neurons using Deep SHAP, as opposed to solely the weights involved.

To further understand the latent space of the classification network, we tested whether there was a dimension that separated between female and male tissue samples. We observed a large statistical difference ($p$ value = $3.6 \times 10^{-249}$) between genders on dimension 78 in the classification model (Figure 5). To understand how dimension 78 captured gender, Deep SHAP was used to explain the genes involved. We found that XIST, the key genes used by the dimensions. As an example, we analyse the highest shared dimension (i.e., dimension 35) in the kidney cancers KIRC, KIRP and KICH (Supplementary Figure S12). APQ2 is the most important gene for that dimension for all three cancer subtypes, which is located in the apical cell membranes of the collecting duct principal cells in kidneys. Additionally, all of the other high ranking genes such as UMOD, SCNN1G and SCNN1B are all well known genes associated with kidney functions [Carney, 2016, Hanukoğlu and Hanukoğlu, 2016]. As another example, we also explain dimension 42 and 73, the $1^{st}$ and $2^{nd}$ most important dimension for lung adenocarcinoma (LUAD) prediction respectively, as shown in Table 4. The top genes were calculated using random training samples as the reference value, to show the most important genes for LUAD versus all the other sample types. We demonstrated that dimension 42 relies heavily on the immune response pathways, whilst dimension 73 relates to the developmental process, albeit with one highly significant immune response pathway. The top gene for dimension 73 is pulmonary-associated surfactant protein C (SPC), a surfactant protein essential for lung function, and the top gene for dimension 42 is progesterone associated endometrial protein (PAEP), an immune system modulator, both of which have been implicated in LUAD [Schneider et al., 2015, Yamamoto et al., 2005].

We found that the most important input features for the latent dimensions varied according to the tumour type used for the analysis (Table 4). This demonstrates a possible limitation of previous methods explaining gene expression classification networks using solely a connection weight approach, for example by Way and Greene [2018] and Bica et al. [2020], which show no specificity for different input samples and different prediction targets. Table 4 shows that for BRCA, dimension 42 uses the genes related to blood vessels, and dimension 73 relies on the embryonic genes. However, this contrasts with the most important pathways that these dimensions used for LUAD classification. XOmivAE is able to capture this as it detects the activation of neurons using Deep SHAP, as opposed to solely the weights involved.

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Influence of important genes for model performance

To further evaluate the results, we compared the classification performance of models using the top 20 XOmivaE genes or 20 random genes for each target tumour type of interest. Four metrics including the F1-score (F1), Positive Predictive Value (PPV), True Positive Rate (TPR) and Area Under the Curve (AUC) were applied, and the performance of models using 20 random genes was averaged over 10 random seeds. A highly significant performance difference can be observed in Table 6, which indicates the contribution of the top genes obtained by XOmivaE to the cancer classification tasks. Additionally, we calculated the average metrics for all the other tumour types except the target one and found that whilst there was also an increase in metrics from the randomly selected genes, it was not as significant as the increase for the target tumour type. This suggests that the top genes revealed by XOmivaE are specific for certain target tumour type.

To approximate the most important genes for the overall model, we summed the ranking of genes for each tumour type, with the most important gene having a ranking of 1 and the least important gene ranking 58,043, and selected 20 genes with the lowest sum rankings to retrain the model and calculate the overall accuracy (Table 7). We then compared it with the performance of a model trained by 20 random genes and a model trained by the overall 20 least important genes with the highest ranking sums. Using the 20 most important genes we observed a significant improvement in accuracy over using a random selection of 20 genes. Additionally, we found that the 20 least important genes caused a large decrease in accuracy compared to a random selection of genes. These results suggest a possible role of using the XOmivaE contribution scores for feature selection in model training with high-dimensional omics data.

Influence of important dimensions for model performance

To understand whether XOmivaE accurately detected the most and least important dimensions in the latent space, we evaluated the effect of knocking out the most important dimensions, Table 8. We set the output of the target dimension to -1 when the output value was positive, and set the output

Table 4. The biological pathways enriched for dimension 42 and 73 when classifying BRCA and LUAD.

| Dimension ID | Tumour type | GO biological process | FDR adjusted p-value |
|--------------|-------------|-----------------------|----------------------|
| 42           | BRCA        | Humoral immune response | 1.8 × 10^{-8}        |
| 42           | BRCA        | Response to bacterium  | 2.0 × 10^{-8}        |
| 42           | BRCA        | Response to stimulus  | 2.0 × 10^{-8}        |
| 42           | BRCA        | Immune system process | 2.5 × 10^{-8}        |
| 42           | BRCA        | Response to other organism | 3.7 × 10^{-8} |
| 42           | BRCA        | Circulatory system process | 4.7 × 10^{-8}      |
| 42           | BRCA        | Blood circulation     | 1.3 × 10^{-8}        |
| 42           | BRCA        | Developmental process | 4.6 × 10^{-8}        |
| 42           | BRCA        | Regulation of blood pressure | 2.0 × 10^{-8} |
| 42           | BRCA        | Humoral immune response | 2.5 × 10^{-8}        |

Table 5. The top five genes for dimension 78 when separating female and male samples in the classification model of OmiVAE.

| Gene       | Contribution score | Chromosome |
|------------|--------------------|------------|
| CLDN3      | 0.00031            | chr7       |
| SLPI       | 0.00031            | chr20      |
| WFDC2      | 0.00031            | chr20      |
| XIST       | 0.00030            | chrX       |
| MMP1       | 0.00029            | chr11      |

Validation of downstream tasks

Influence of important genes for model performance

Highly significant performance difference can be observed in Table 6, which indicates the contribution of the top genes obtained by XOmivaE to the cancer classification tasks. Additionally, we calculated the average metrics for all the other tumour types except the target one and found that whilst there was also an increase in metrics from the randomly selected genes, it was not as significant as the increase for the target tumour type. This suggests that the top genes revealed by XOmivaE are specific for certain target tumour type.

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Table 6. The evaluation metrics of cancer classification using only the top 20 genes obtained by XOmiVAE (columns 1 and 3) or 20 random genes chosen from the overall gene set of 58,043 features (columns 2 and 4). The metrics for each individual tumour type of interest are shown in columns 1 and 2, and the metrics for all of the other tumour types (except the target one) are shown in columns 3 and 4. The results were averaged among all 33 target tumour types and 10 random seeds.

| Table 7. The accuracy of XOmiVAE using the full gene set, the top 20 contribution genes for all tumours, 20 random genes and the bottom 20 contribution genes for all tumours. |
|---|---|---|---|
| Gene set | N | Overall accuracy |
| Full gene set | 58,043 | 96.85% ± 0.46% |
| Top 20 genes for all tumours | 20 | 87.07% ± 0.38% |
| 20 random genes | 20 | 56.10% ± 0.24% |
| Bottom 20 genes for all tumours | 20 | 1.68% ± 0.37% |

| Table 8. The accuracy difference for each tumour type when the most important and least important dimensions were individually or together removed from the network. Values represent the mean and standard deviation of the accuracy difference among 33 tumour types. |
|---|---|
| Ablated dimension | Accuracy change |
| 1st | −11.9% ± 19.9% |
| 2nd | −11.9% ± 20.2% |
| 3rd | −2.9% ± 6.5% |
| Top three combined | −95.9% ± 2.0% |
| 126th | 0.0% ± 0.3% |
| 127th | 0.0% ± 0.0% |
| 128th | 0.0% ± 0.3% |
| Bottom three combined | 0.0% ± 0.3% |

of the target dimension to 1 when the output value was negative, based off a similar ablation approach by Morcos et al. [2018]. This ensures that the output is perturbed from the original value. Individually, the most important dimensions did not have a large effect when ablated, which is likely due to model saturation, a feature of neural networks that Deep SHAP addresses whereas other interpretability techniques fail to capture [Shrikumar et al., 2017]. When the top dimensions combined were ablated, the classification accuracy fell to 0. This is in contrast to the least important dimensions, which did not have any effect on the network when knocked out, individually or combined. This provides evidence to support the most and least important dimensions obtained by XOmiVAE.

Explaining unsupervised clustering results

As an example of explaining the unsupervised clustering results, we used Basal-like (Basal) and Luminal B (LumB) breast tumour subtypes. Explaining the latent dimensions of VAEs would be crucial when it is important to understand the genes involved in subtype clustering of cancers that are yet to be defined, and labels that could be used for supervised learning are scarce. Figure 7 shows the two most decisive dimensions splitting the subtypes. As the most statistically significant dimension for separating the two subtypes was dimension 100, we evaluated the enriched pathways when this dimension is used to separate Basal and LumB. Here, the \( \mu \) value for a subtype (LumB) was treated as the output and backpropagated through the network using Deep SHAP, and compared to the other subtype (Basal) as the reference.
Fig. 6. The top 15 genes obtained by XOmiVAE for the classification of BRCA using normal breast tissue samples as the reference. As we were interested in validating whether the model can explain the subtype specific pathways, we evaluated the top 100 genes using the Broad Institute’s curated pathway database [Subramanian et al., 2005], which includes pathways from experiments comparing the subtypes.

In Table 9 we can see the pathways are highly specific for the subtypes. A key differentiating feature between the subtypes is that LumB is estrogen-receptor (ESR1) positive, and Basal is ESR1 negative and in Table 9 we can see the top pathways also include the genes that differentiate between the ESR1 negative and ESR1 positive tumours. Table 10 shows the results when the three other BRCA subtypes (LumA, Her2 and Basal) are used as the reference samples when explaining subtype LumB. The results show that a larger range of subtype pathways are present in the most important features. These results proves that it is a useful method of being able to obtain the unique genes for one subtype versus multiple other subtypes.

This is, to the best of our knowledge, the first attempt at using an activation level-based explanation approach for clustering generated by autoencoders. Typically, differential gene expression methods, such as DESeq2 [Love et al., 2014], is used to explain differences in clusters, which treats each gene as independent. More recent methods improve on this, such as Global Counterfactual Explanation (GCE) [Plumb et al., 2020] and Gene Relevance Score (GRS) [Angerer et al., 2020]. However, GCE requires a linear embedding, and the embedding of GRS is constrained to ensure the gradients are easy to calculate. XOmiVAE requires a non-linear embedding, and becomes one of the first activated-based deep learning interpretation method to explain novel clusters generated by VAEs.

Conclusion
Here we presented an explainable variational autoencoder based deep learning method for high dimensional omics data analysis, named XOmiVAE. We illustrated that it is possible to explain the supervised task of the network and obtain the most important genes and dimensions for a prediction. We also showed that it is practicable to explain the most important genes in an unsupervised network, and therefore provide a method for explaining deep learning based clustering. We evaluated the explanations of XOmiVAE and demonstrated that they make biological sense. Additionally, we offered important steps to consider when interpreting deep learning models for tumour classification. For example, we highlighted the importance of choosing reference samples that makes biological sense when explaining the model, and we disclosed the limitations of connection weight based methods to explain latent dimensions. We believe XOmiVAE is a promising methodology that could help open the “black box” and discover novel biomedical knowledge from deep learning models.

Key Points
- XOmiVAE is a novel interpretable deep learning model for cancer classification using high-dimensional omics data.
- XOmiVAE provides contribution score of each input molecular feature and omics latent dimension for each prediction.
- XOmiVAE is able to explain unsupervised clusters produced by VAEs without the need for labeling.
- XOmiVAE explanations of the downstream prediction were evaluated by biological annotation and literature, which aligned with current domain knowledge.
- XOmiVAE shows great potential for novel biomedical knowledge discovery from deep learning models.

Availability
The source code have been made publicly available on GitHub https://github.com/zhangxiaoyu11/XOmiVAE/. The TCGA pan-cancer dataset can be downloaded from the UCSC Xena data portal https://xenabrowser.net/datapages/.

Supplementary data
Supplementary is available at GitHub https://github.com/zhangxiaoyu11/XOmiVAE/blob/main/documents/supplementary.pdf.

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Table 9. The top pathways for differentiating LumB and Basal (BRCA subtypes), using the Broad Institute’s curated pathway database.

| Pathway                                                                 | Genes in overlap | P-value        |
|------------------------------------------------------------------------|-----------------|----------------|
| Genes up-regulated in breast cancer samples positive for ESR1 compared | 27              | 1.15e-47       |
| to the ESR1 negative tumours                                           |                 |                |
| Genes down-regulated in basal subtype of breast cancer samples         | 39              | 4.25e-43       |
| Genes up-regulated in bone relapse of breast cancer                    | 24              | 2.6e-42        |
| Genes which best discriminated between two groups of breast cancer      | 29              | 1.85e-37       |
| according to the status of ESR1 and AR basal (ESR1- AR-) and luminal   |                 |                |
| (ESR1+ AR+)                                                            |                 |                |
| Genes up-regulated in luminal-like breast cancer cell lines compared    | 26              | 1.82e-30       |
| to the basal-like ones                                                  |                 |                |

Table 10. The top pathways for differentiating between LumB and the other three subtypes (Basal, LumA and Her2), using the Broad Institute’s curated pathway database.

| Pathway                                                                 | Genes in overlap | P-value        |
|------------------------------------------------------------------------|-----------------|----------------|
| Genes down-regulated in basal subtype of breast cancer samples         | 27              | 1.15e-47       |
| Genes up-regulated in bone relapse of breast cancer                    | 39              | 4.25e-43       |
| Genes down-regulated in ductal carcinoma vs normal ductal breast cells | 24              | 2.6e-42        |
| Genes down-regulated in nasopharyngeal carcinoma (NPC) positive for   | 29              | 1.85e-37       |
| LMP1, a latent gene of Epstein-Barr virus (EBV)                        |                 |                |
| Genes up-regulated in breast cancer samples positive for ESR1 compared | 26              | 1.82e-30       |
| to the ESR1 negative tumours                                           |                 |                |

Competing interests

The authors declare that they have no conflict of interest.

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Eloise Withnell is currently a PhD candidate at Department of Health Informatics, University College London, London, UK.

Xiaoyu Zhang is currently a PhD candidate at Data Science Institute, Imperial College London, London, UK.

Kai Sun is currently the acting operations manager of Data Science Institute, Imperial College London, London, UK.

Yike Guo is currently the co-director of Data Science Institute, Imperial College London, London, UK and vice-president of Hong Kong Baptist University, Hong Kong, China.