Evaluating deep variational autoencoders trained on pan-cancer gene expression

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Summary

Cancer is a heterogeneous disease with diverse molecular etiologies and outcomes. The Cancer Genome Atlas (TCGA) has released a large compendium of over 10,000 tumors with RNA-seq gene expression measurements. Gene expression captures the diverse molecular profiles of tumors and can be interrogated to reveal differential pathway activations. Deep unsupervised models, including Variational Autoencoders (VAE) can be used to reveal these underlying patterns. We compare a one-hidden layer VAE to two alternative VAE architectures with increased depth. We determine the additional capacity marginally improves performance. We train and compare the three VAE architectures to other dimensionality reduction techniques including principal components analysis (PCA), independent components analysis (ICA), non-negative matrix factorization (NMF), and analysis of gene expression by denoising autoencoders (ADAGE). We compare performance in a supervised learning task predicting gene inactivation pan-cancer and in a latent space analysis of high grade serous ovarian cancer (HGSC) subtypes. We do not observe substantial differences across algorithms in the classification task. VAE latent spaces offer biological insights into HGSC subtype biology.

1 Introduction

From a systems biology perspective, the transcriptome can reveal the overall state of a tumor [1]. This state involves diverse etiologies and aberrantly active pathways that act together to produce neoplasia, growth, and metastasis [2]. Such patterns can be extracted with machine learning.

Deep generative models have improved state of the art in several domains including image and text processing [3–5]. Such models can simulate realistic data by learning an underlying data generating manifold. The manifold can be mathematically manipulated to extract interpretable elements from the data. For example, a recent imaging study subtracted the vector representation of a “neutral woman” from a “smiling woman”, added the result to a “neutral man”, and revealed image vectors representing “smiling men” [6]. In text processing, $\vec{\text{king}} - \vec{\text{man}} + \vec{\text{woman}} = \vec{\text{queen}}$ [7].

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Previously, nonlinear dimensionality reduction approaches have revealed complex patterns and novel biology from publicly available gene expression data \cite{8,9}, including drug response predictions \cite{10}. Here, we extend a one-hidden layer VAE model, named Tybalt \cite{11}. We train and evaluate Tybalt, plus two other VAE architectures and compare them to other dimensionality reduction algorithms.

2 Methods

2.1 The Cancer Genome Atlas RNAseq Data

We used TCGA PanCanAtlas RNA-seq data from 33 different cancer-types \cite{12}. The data includes 10,459 samples (9,732 tumors and 727 tumor adjacent normal). The data was batch corrected and RSEM preprocessed, and is in the log2(FPKM + 1) format. To facilitate VAE training, we zero-one normalized by gene. We also used zero-one normalization for NMF and ADAGE. We used z-scored data for PCA and ICA. All data are publicly available and were accessed from the UCSC Xena browser under a versioned Zenodo archive \cite{13}.

2.2 Variational Autoencoder Training

We trained VAE models as previously described \cite{11}. We performed a grid search over hyperparameters and defined optimal models by lowest holdout validation loss. VAE loss is the sum of reconstruction loss and a Kullback-Leibler (KL) divergence term constraining feature activations to a Gaussian distribution \cite{3,4}. We searched over batch sizes, epochs, learning rates, and kappa values. Kappa controls “warmup”, which determines how quickly the loss term incorporates KL divergence \cite{14}. VAEs learn two distinct latent representations, a mean and a standard deviation vector, which are reparametrized into a single vector that can be back-propagated. This enables rapid sampling over features to simulate data. We trained our models using Keras \cite{15} with a TensorFlow backend \cite{16}. We trained and evaluated the performance of three VAE architectures (Table 1). Using an EVGA GeForce GTX 1060 GPU, all VAE models were trained in under 4 minutes.

| Name              | Hidden Layers | Hidden Layer Size | Latent Feature Size |
|-------------------|---------------|-------------------|--------------------|
| Tybalt            | 1             |                   | 100                |
| Two Hidden VAE (100) | 2             | 100               | 100                |
| Two Hidden VAE (300) | 2             | 300               | 100                |

2.3 Dimensionality Reduction Analysis

We evaluated the ability of the VAE models to generate biologically meaningful features. We compared these features to PCA, ICA, NMF, and ADAGE features derived from the same pan-cancer RNAseq data.

2.3.1 Predicting NF1 inactivation in tumors from gene expression data

Molecular aberrations in cancer form gene expression signatures that supervised machine learning algorithms can detect \cite{17,18}. We trained elastic net logistic regression classifiers to detect pan-cancer tumors with inactivated NF1. We defined NF1 inactivated tumors based on the presence of deleterious NF1 mutations or NF1 deep copy number loss. NF1 inactivation is difficult but important to detect because NF1 can be inactivated by multiple mechanisms and reliable classification is required for effective targeted therapies \cite{19}. We trained independent models using pan-cancer RNAseq features derived from each dimensionality reduction algorithm. We report performance during 5-fold cross validation. We performed all training and evaluation using sci-kit learn \cite{20}. We trained our models using tumors that had matched mutation, copy number, and RNAseq data, and we used only cancer-types that had greater than 10 NF1 inactivated samples (n = 1774). This included bladder carcinoma (BLCA), low grade glioma (LGG), lung adenocarcinoma (LUAD), paraganglioma and pheochromocytoma (PCPG), skin cutaneous melanoma (SKCM), and stomach adenocarcinoma (STAD). As a negative control, we shuffled gene expression profiles for each sample independently, and used these shuffled matrices for predictions.
2.3.2 High grade serous ovarian cancer arithmetic

Four HGSC subtypes have been previously described [21]. However, they are not consistent across populations [22]. Using original TCGA subtype labels [23], we calculated mean HGSC subtype vector representations for the mesenchymal and immunoreactive subtypes with the latent space representation from each dimensionality reduction algorithm. Samples from the two subtypes were often aggregated under different clustering initializations [22]. The mesenchymal subtype is defined by poor prognoses, overexpression of extracellular matrix genes, and increased desmoplasia, while the immunoreactive subtype displays increased survival and immune cell infiltration [24, 25]. We hypothesized that subtracting latent space representations of subtypes would reveal biological patterns. To characterize the patterns, we performed pathway overrepresentation analyses (ORA) [26] over Gene Ontology (GO) terms [27] using the high weight genes (> 2.5 standard deviations from the mean) of the highest differentiating positive feature. We report the most significantly overrepresented GO term.

3 Results

3.1 Modest improvements by architecture

We observed increased performance for the two-hidden layer models, and an additional increase for the model with two compression steps. However, the benefits were modest as compared to the one-hidden layer Tybalt model (Figure 1).

3.2 Dimensionality Reduction Comparison

3.2.1 Supervised classification of NF1 inactivation

Based on receiver operating characteristic (ROC) curves, all algorithms had relatively similar performance (Figure 2A). PCA performed slightly better than other dimensionality reduction algorithms (area under the ROC curve (AUROC) = 65.6%). However, raw gene expression features performed the best (AUROC = 68.4%). There were more classification features used for models built with raw features as compared to other methods; NMF included many features where PCA included the fewest (Figure 2B). The shuffled dataset contained the highest number of features, and was somewhat predictive of NF1 status (AUROC = 55.4%), which may be an artifact of relatively low sample sizes.

3.2.2 Latent space arithmetic of HGSC subtypes

We ranked mean activation differences across dimensionality reduction methods (Figure 3). PCA included the largest number of features with high values, while ICA and NMF had few activation differences. The nonlinear neural network based approaches displayed sparse feature differences. ORA analyses revealed few significant terms in the linear methods (Table 2). Of the nonlinear methods, Tybalt, and the 300-hidden node VAE both identified a collagen term, which is known to be associated with mesenchymal subtype tumors [24].
Figure 2: Evaluating dimensionality reduction algorithms in predicting NF1 inactivation pan-cancer. (A) Receiver operating characteristic curve for cross validation intervals. (B) Number of features selected by each model. The colors are consistent between figure panels.

Figure 3: Subtracting mean vector representations of the Mesenchymal and Immunoreactive High Grade Serous Ovarian Cancer (HGSC) subtypes.

Table 2: Dimensionality reduction algorithms subtraction comparison

| Algorithm | Top Pathway                                      | Adj. p value |
|-----------|--------------------------------------------------|--------------|
| PCA       | Zero high weight genes                           |              |
| ICA       | No significant pathways                          |              |
| NMF       | Homophilic Cell Adhesion Via Plasma Membrane Adhesion Molecules | 3.3e−06      |
| ADAGE     | No significant pathways                          |              |
| Tybalt    | Collagen Catabolic Process                       | 1.8e−09      |
| VAE (100) | Epidermis Development                            | 8.0e−04      |
| VAE (300) | Collagen Catabolic Process                       | 1.7e−03      |

4 Conclusions

We evaluated the performance of three VAE models trained on TCGA pan-cancer gene expression. While we did not explore larger architectures with higher capacity, it appears that increasing the depth of model only modestly improves performance. It is also likely that increasing model depth reduces the ability to interpret the model. We also did not compare performance across different sized latent spaces. Nevertheless, we show that VAEs capture signals that are able to predict gene inactivation comparably to other algorithms. We demonstrate that the VAE latent space arithmetic provides a unique benefit and should be explored further in the context of gene expression data. We provide source code to reproduce training and latent space analyses at https://github.com/greenelab/tybalt [28] and pan-cancer classifier analyses at https://github.com/greenelab/pancancer [29].

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