MLACP 2.0: An updated machine learning tool for anticancer peptide prediction

Le Thi Phan à,¹, Hyun Woo Park à,¹, Thejkiran Pitti à, Thirumurthy Madhavan c, Young-Jun Jeon b,²,*, Balachandran Manavalan à,³,⁴

¹Computational Biology and Bioinformatics Laboratory, Department of Integrative Biotechnology, College of Biotechnology and Bioengineering, Sungkyunkwan University, Suwon 16419, Gyeonggi-do, Republic of Korea
²Department of Integrative Biotechnology, College of Biotechnology and Bioengineering, Sungkyunkwan University, Suwon 16419, Gyeonggi-do, Republic of Korea
³Computational Biology Lab, Department of Genetic Engineering, SRM Institute of Science & Technology, Kattankulathur 603203, Tamil Nadu, India

Article info
Article history:
Received 18 April 2022
Received in revised form 25 July 2022
Accepted 25 July 2022
Available online 2 August 2022

Keywords:
Anticancer peptides
Convolutional neural network
Feature encodings
Conventional classifiers
Baseline models
Dataset construction

Abstract
Anticancer peptides are emerging anticancer drug that offers fewer side effects and is more effective than chemotherapy and targeted therapy. Predicting anticancer peptides from sequence information is one of the most challenging tasks in immunoinformatics. In the past ten years, machine learning-based approaches have been proposed for identifying ACP activity from peptide sequences. These methods include our previous method MLACP (developed in 2017) which made a significant impact on anticancer research. MLACP tool has been widely used by the research community, however, its robustness must be improved significantly for its continued practical application. In this study, the first large non-redundant training and independent datasets were constructed for ACP research. Using the training dataset, the study explored a wide range of feature encodings and developed their respective models using seven different conventional classifiers. Subsequently, a subset of encoding-based models was selected for each classifier based on their performance, whose predicted scores were concatenated and trained through a convolutional neural network (CNN), whose corresponding predictor is named MLACP 2.0. The evaluation of MLACP 2.0 with a very diverse independent dataset showed excellent performance and significantly outperformed the recent ACP prediction tools. Additionally, MLACP 2.0 exhibits superior performance during cross-validation and independent assessment when compared to CNN-based embedding models and conventional single models. Consequently, we anticipate that our proposed MLACP 2.0 will facilitate the design of hypothesis-driven experiments by making it easier to discover novel ACPs. The MLACP 2.0 is freely available at https://balab-skku.org/mlacp2.

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1. Introduction
Cancer is one of the prominent threats to human health, and it is often linked to a higher mortality rate as life expectancy increases, both in developed and developing countries [1]. In 2018, the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) reported that 18.1 million new cases and 9.6 million deaths were caused by cancer [2]. Cancer results from the uncontrolled proliferation of abnormal cells which invade normal tissues and organs and multiply in an uncontrolled manner [3]. The complexity and heterogeneity of cancer make its treatment difficult. Hence, cancer therapy must focus primarily on limiting the proliferation of cancer cells and inhibiting their spread [4].

With traditional surgery, precise removal of the cancerous part could not be guaranteed [5]. Radiotherapy, chemotherapy, and targeted therapy are the most common treatment options for cancer. In spite of this, these therapies are not very precise, as they fail to differentiate cancer cells from healthy cells and result in both damaging healthy cells and killing cancer cells. However, these therapies are expensive and have negative side effects on patients [6,7]. Furthermore, cancer cells can develop resistance to chemotherapy drugs due to the fact that their genomes are changing dynamically [8]. Consequently, there is an urgent need to develop a novel cancer treatment that is free of adverse effects, reduces drug resistance, and specifically targets cancer cells.
Antimicrobial peptides (AMPs) are a diverse class of bioactive molecules that provide protection against bacteria, protozoa, fungi, and viruses [9]. A subset of AMPs exhibiting potential anticancer properties is referred to as anticancer peptides (ACPs), which are short peptides whose sequence length does not exceed 50 amino acid residues [10]. ACPs possess amphiphilic properties due to the presence of hydrophobic and positive residues that interact with the anionic membranes of cancer cells, selectively targeting and killing them [11]. ACPs can target cancer cells based on the membrane charge that forms an electrostatic interaction between the membrane and the ACPs, thus leaving normal cells untouched. This is the major advantage of using ACPs over currently available approaches in cancer treatment [12,13]. Moreover, ACPs are naturally biological inhibitors, and they are also easy to synthesize, which makes them an ideal therapeutic agent to treat cancer [13]. In light of this, peptide-based therapeutics have emerged as a promising therapeutic agent for treating cancer compared to conventional therapies because they are less toxic, highly specific, capable of penetrating membranes, and easy to modify chemically [14,15].

Several computational predictors have been proposed for the identification of ACPs. Many of these methods have been reviewed in recent literatures [16,17], including our previous method, MLACP [18]. It was built using an imbalanced dataset, a linear integration of four different encodings, a support vector machine (SVM), and a random forest (RF). The MLACP is widely used among the research community, thus gaining popularity within the ACP research community. The number of experimentally verified ACPs is increasing exponentially; therefore, it is high time to update the previous version utilizing advanced computational techniques, thus increasing its accuracy and robustness.

The development of MLACP 2.0 involves the following steps: (i) Created a high-quality non-redundant training dataset and independent datasets based on extensive literature/database searches. (ii) Systematically evaluated 17 different feature encodings (including both conventional encodings and word embeddings) and built the corresponding model using seven different conventional classifiers (RF, gradient boosting (GB), SVM, extreme gradient boosting (XGB), AdaBoost (AB), light gradient boosting (LGB), and extremely randomized tree (ERT)). (iii) For each classifier, choose a subset of models based on certain criteria from 17 encoding-based models. (iv) Calculate the probability of ACPs from the seven classifiers based on a subset of selected models was concatenated and trained using a convolutional neural network (CNN) for the final prediction, MLACP 2.0. Extensive benchmark experiments demonstrate the effectiveness of MLACP 2.0: it achieves a more accurate and stable performance compared with conventional single-encoding models and CNN-based one-hot encoding and word embedding models, on both the basis of cross-validation and independent assessment. On an independent test, MLACP 2.0 significantly outperformed the existing predictors. Using the proposed hybrid ensemble model, a user-friendly online predictor of MLACP (https://balalab-skku.org/mlacp2/) is implemented.

Notably, several methods used the same datasets, and therefore some sequences are redundant. A CD-HIT [19] of 0.8 was applied among ACPs, which resulted in 1084 peptide sequences. The same cut-off was applied to non-ACP samples whose sequences overlapping with ACPs resulted in ~ 7500 sequences. However, we randomly selected 1084 non-ACPs to avoid class bias during the model building and balanced the ACPs. This is the first time such a large non-redundant dataset has been used for training or model building in ACP prediction research.

**Independent dataset:** ACPs were extracted from the following 11 databases, CancerPPD [20], APD3 [21], PlantPepDB [22], DBAASP v3.0 [23], SATPdb [24], ADAM (https://bioinformatics.cs.ntou.edu.tw/ADAM), DRAMP 3.0 [25,26], LAMP [27], Peptipedia [28], DbAMP [29], and AMPfun [30], resulting in 3725 ACPs. Secondly, a CD-HIT of 0.80 was applied to the collected ACPs that overlapped with the training ACP sequences, resulting in 769 sequences. Unlike previous studies, where random peptides were considered as non-ACPs, we considered other functional peptides (antihypertensive and antiviral, etc.), a small portion of random peptides, ACPs, and non-ACPs, and experimentally confirmed non-inflammatory inducing peptides as non-ACPs, resulting 1287 non-redundant non-ACPs. This independent dataset can be used as a gold standard for evaluating future ACP prediction models. Furthermore, the supplementary information includes a brief description of the dataset length distribution and compositional analysis.

### 2.2. Feature encodings

The process of exploring different feature encodings on the same dataset is essential to understand and identify the appropriate encodings. Keeping this in mind, a wide range of features were used in this study, including 15 conventional encodings (dipeptide composition (DPC), dipeptide deviation from the expected mean (DDE), amino acid composition (AAC), composition transition and distribution (CTDC, CTDT, and CTDD), grouped DPC (GDPC), enhanced grouped AAC (EGAAC), grouped tripeptide composition (GTPC), BloSUM62 (BLOS), enhanced AAC (EAAC), K-spaced con-joint triad (KSC), quasi sequence order (QSO) composition of K-spaced amino acid group pairs (KSAAGP), and Z scale) and two-word embeddings are one-hot encoding (1OHE) and pretrained embedding from seq2vec. Among these 17 encodings, 11 encodings (AAC, KSAAGP, CTDC, CTDT, CTDD, DDE, DPC, KSC, QSO, 1OHE, and seq2vec) are the most important and contributed significantly to the ACP prediction. Notably, nine of the conventional encodings contributed to the final prediction, whose encoding details are extensively described in our previous studies [31].

Using the same procedure AAC, KSAAGP, CTDC, CTDT, CTDD, DDE, DPC, KSC, QSO encoded 20, 195, 395, 39, 400, 400, 343, 343, 100 D feature vectors, respectively. Notably, these features have been normalized as follows: $X_{norm} = \frac{x - \min(x)}{\max(x) - \min(x)}$. A brief description of these word embeddings is as follows:

### 2.3. 1OHE

The one-hot encoding method is quite popular among binary encoding techniques. The maximum length of peptides in our dataset is 50 amino acids. If the residues are<50 amino acids, a dummy residue X is added to the C-terminus. Therefore, each amino acid is represented by a 21-dimensional feature vector, where the standard amino acid is characterized by 1 at various positions and zero at the remaining 20 positions. Dummy residues, on the other hand, consist entirely of zeros. This resulted in a 1050-D feature vector.
2.4. Seq2vec

We utilized seq2vec’s pretrained embeddings to achieve the concept of transfer learning. Heinzinger et al. developed pretrained embeddings by training the ELMo model using millions of protein sequences extracted from UniRef50. For this study, we used the same pretrained embeddings that provide a 1024 D feature vector for a given peptide sequence.

2.5. MLACP 2.0 framework

The MLACP 2.0 framework (Fig. 1) was developed using the training dataset and feature encodings mentioned above, and it consists of constructing baseline models and developing a meta-predictor.

Construction of baseline models: We utilized seven different classifiers (RF, ERT, SVM GB, AB, LGB, and XGB) that have been extensively applied in Bioinformatics and computational biology [32–37]. For each classifier, there are a set of hyperparameters that determine the performance of the model during cross-validation. We optimized the hyperparameters using a grid search approach and 10-fold cross-validation. To construct each baseline model, 10-fold cross-validation was repeated five times with random partitioning of the training samples, and the median parameters were taken as the final optimal values. These values were then used to construct the final baseline model. The hyperparameters search range for each classifier is as follows: (i) LGB seven hyperparameters are: num_leaves ∈ [50 to 1000] with an interval of 20, max_bins ∈ [200 to 400] with an interval of 10, n_estimators ∈ [100 to 2000] with an interval of 10, min_child_samples ∈ [30 to 400] with an interval of 10, max_depth ∈ [5 to 12] with an interval of 1, learning_rate ∈ [10^{-6} to 10^{-1}], and bagging fraction is 0.8. (ii) RF and ERT have the same hyperparameters with different model construction procedures, whose search ranges are: n_estimators ∈ [50, 75, ..., 3000], max_features ∈ [1, 2, ..., 20], and min_samples_split ∈ [2, 3, ..., 10]. (iii) SVM two hyperparameters are C ∈ [2^{-15}, 2^{15}] with a step size of 2 and γ ∈ [2^{-13}, 2^{3}] with a step size of 2^{-3}. (iv) AB three hyperparameters are: n_estimators ∈ [10, 20, ..., 500], max_depth ∈ [1, 2, ..., 11], and learning_rate ∈ [1, 0.5, 0.25, 0.1, 0.05, 0.01]. (v) GB hyperparameters search ranges are n_estimators ∈ [10, 20, ..., 500], learning_rate ∈ [1, 0.5, 0.25, 0.1, 0.05, 0.01], and max_features ∈ [1, 2, ..., 10]. (vi) four XGB hyperparameters are: n_estimators ∈ [10, 20, ..., 2000], max_depth ∈ [1, 2, ..., 11]; learning_rate ∈ [1.0, 0.5, 0.25, 0.1, 0.05, 0.01, 0.001, 0.02, 0.3], and eta ∈ [0.0001, 0.001, 0.002, 0.01, 0.02, 0.05, 1.0]. Notably, the names of hyperparameters are derived from those given in their corresponding package. This work implemented the scikit-learn version 0.24.2 [38] library for five classifiers (SVM, RF, GB, AB, and ERT) lightGBM version 3.3.0 [39], and XGBoost version 0.82 python package to carry out the classification task.

Finally, a total of 119 baseline models were generated, and a set of models was selected based on the following criteria: (i) computed cumulative score (CS) of Mathews correlation coefficient, accuracy (ACC), and area under the ROC curve (AUC) for each baseline model. (ii) selected a set of baseline models for each classifier, whose CS is greater than the mean CS. Consequently, this filtering resulted in 67 baseline models. The predicted probability values of...
the selected models (67D) are used for the development of the meta predictor.

Construction of meta-predictor: A similar CNN network architecture was adopted based on recent studies [40,41]. However, we optimized four filters, epochs, and batch size using 10-fold cross-validation. Specifically, the predicted probability of 67D feature vector input to four 1D convolutional layers. The kernel sizes associated with these layers are 4, 5, 6, and 7, with corresponding filters of 20, 8, 32, and 8, respectively. After the convolutional operation, the activation function of the rectified linear unit (ReLU) was applied, which can be described as follows:

\[
\text{ReLU}(x) = \max(0, x) = \begin{cases} 
  x & \text{if } x > 0 \\
  0 & \text{else} 
\end{cases}
\]

For each of the four 1D convolutional layers, 20, 8, 32, and 8 feature maps were generated. Following each 1D convolutional layer, a 1D Global Max Pooling layer was applied, whose purpose was to find the maximum values from the feature maps that were converted to univariate feature vectors and concatenated subsequently. Additionally, a dropout rate of 0.5 is used for the independent component layer. Then, three dense layers of 32, 16, and 8 neurons, respectively, were applied with the ReLU activation function. Finally, a dense layer composed of a single neuron with a sigmoid function is applied, which produces a value between 0 and 1. If the value is greater than 0.5, the peptide belongs to ACP, otherwise non-ACP. Adam optimization was used to update the network weights. Notably, CNN was implemented using Keras deep learning library [42].

Sigmoid(x) = \frac{1}{1 + e^{-x}}

2.6. Model evaluation

Furthermore, we considered the commonly used six evaluation metrics to evaluate the model performance [16,43], including MCC, Sensitivity (Sn), Specificity (Sp), ACC, and AUC. The definition of the metrics is as follows:

\[
\begin{align*}
\text{Sn} & = \frac{TP}{TP+FN} \\
\text{Sp} & = \frac{TN}{TN+FP} \\
\text{ACC} & = \frac{TP+TN}{TP+TN+FP+FN} \\
\text{MCC} & = \frac{(TP\cdot TN) - (FP\cdot FN)}{\sqrt{(TP+FN)(TP+FP)(TN+FN)(FN+TN)}}
\end{align*}
\]

where TP, TN, FP, FN, respectively denote true positives, true negatives, false positives, and false negatives. Furthermore, ROC curves and AUC values were used to assess overall performance.

3. Results and discussion

3.1. Construction of baseline models

This study utilized 17 different encodings (QSO, seq2vec, AAC, DPC, CTDC, CTDD, CTD, CKSAAGP, KSC, DDE, 1OH, EAA, GTPC, BLOS, ZSC, GPDC, and EGAAC) that mostly covered composition, physicochemical properties, evolutionary information, word embedding, and position-specific information. The discriminative ability of each of these encodings was evaluated by seven different conventional classifiers (RF, ERT, GB, AB, SVM, LGB, and XGB). Table S1-S7 shows the performances of these encodings to each classifier. Result shows that RF, ERT, GB, XGB, LGB, AB, and SVM have ACC ranges of 72.0–83.0 %, 72.0–83.1 %, 71.3–83.5 %, 70.6–82.5 %, 70.0–83.0 %, 71.7–82.8 %, and 71.1–83.1 %, respectively. We observed that roughly 10 % ACC gap between 17 baseline models produced by each classifier. The QSO encoding achieved the best performance based on four classifiers and the seq2vec encoding achieved the best performance based on two classifiers (XGB and LGB). Generally, most ACP predictors demonstrated high accuracy of >90.0 % in training datasets that contained homologous sequences with high similarity [44,45]. While the best baseline model (QSO-GB) achieved the maximum ACC of 83.5 %, it indicates a drop in performance due to the highly non-redundant training dataset, which may limit the overestimation of model performance.

3.2. Construction of MLACP 2.0

It is likely that considering the 119 baseline models (17 baseline models × 7 classifiers) for the final model construction is not appropriate due to the performance gap of (~10 %) for each classifier among 17 baseline models. Therefore, we calculated the average MCC from 17 baseline models for each classifier and then considered only models whose performance was above average MCC, resulting in 10 baseline models respectively from RF, GB, LGB, and XGB, and 9 respectively from ERT, SVM, and AB. In total, we obtained 67 baseline models, whose performance is shown in Fig. 2, whose ACC is in the range of 0.800–0.835. Next, we examined how many unique encodings contributed to the 67 baseline models. Out of 17 encodings, only 11 encodings (AAC, CKSAAGP, CTDC, CTDD, CTD, DDE, DPC, KSC, QSO, seq2vec, and 10HE) contributed to the selected baseline models, which are mostly composition-based and word embeddings. The remaining six encodings (EAA, EGAAC, GTPC, GDPC, ZSC, and BLOS) based models were excluded because of their relatively lower performance during the training. Based upon the selected baseline models, the prediction probability of ACPs is concatenated and treated as a novel feature vector, which is then trained with CNN to develop the final prediction model. Notably, we also tested with the other seven classifiers employed for the baseline model construction, but CNN has the edge in terms of robustness (Figure S8).

Hence, we selected CNN for the meta-model construction, named MLACP 2.0. It achieved MCC, ACC, Sn, Sp, and AUC of 0.694, 0.846, 0.815, 0.876, and 0.915, respectively.

3.3. Comparison of MLACP 2.0 with different approaches to training dataset

For the purpose of illustrating the advantages of using probabilistic features in MLACP 2.0, we have also developed CNN-based word embedding models, namely seq2vec-CNN and 10HE-CNN, as well as a CNN model based on hybrid features (a linear integration of eleven encodings). Fig. 3 compares the performance of MLACP 2.0 with the top five baseline models, CNN-based word embeddings, and hybrid feature models. Compared to the best five baseline models, the CNN-hybrid model performs similarly to the best five baseline models, and significantly better than the CNN-word embedding model, indicating that automated word embedding features are not as effective as feature engineering in ACP prediction. MLACP 2.0 outperforms the best five baseline models as well as CNN-based models. More specifically, the improvements of MLACP 2.0 are 2.3–10.3 % in MCC, 1.1–5.2 % in ACC, and 0.5–4.8 % in AUC, demonstrating that a systematic approach to evaluating multiple encodings in tandem with the selection of a set of baseline models utilized for meta-model construction led to improved performance.

3.4. Evaluation of MLACP 2.0 and the state-of-the-art methods on an independent dataset

The independent dataset was used to evaluate MLACP 2.0 along with the previous version and the two best ACP predictors.
Notably, two of the methods were reported to be the best predictors in previous studies by unbiased evaluations. In contrast to the routine independent data-sets used in previous studies, a challenging dataset was created. Independent datasets have the following important characteristics: (i) none of the ACPs share >80 % sequence identity with the training dataset; and (ii) the non-ACPs were constructed considering several practical scenarios, including other functional peptides and experimentally characterized negative examples. According to Table 1, MLACP 2.0 achieves MCC, ACC, Sn, Sp, and AUC values of 0.513, 0.765, 0.750, and 0.773, and 0.817, respectively. In particular, MLACP 2.0 improved the MCC of 16.2–28.0 %, the ACC of 0.8–13.7 %, and the AUC of 7.3–17.7 % compared to the existing predictors. Furthermore, MLACP 2.0 predictor performance is more balanced (low difference between Sn and Sp) when compared to the existing predictors, demonstrating that MLACP 2.0 performs well on unseen data and is better suited for practical applications.

**Fig. 2.** Performance comparison of the baseline models selected for each classifier. (A) random forest (RF), (B) gradient boosting (GB), (C) extremely randomized tree (ERT), (D) light gradient boosting (LGB), (E) extreme gradient boosting (XGB), (F) AdaBoost (AB), and (G) support vector machine (SVM).

**Fig. 3.** Performance comparison of MLACP 2.0 with the top five baseline models and with other approaches based on the training dataset.

**Table 1** Performance of different methods on independent datasets.

| Methods             | MCC   | Accuracy | Sensitivity | Specificity | AUC   | P-value   |
|---------------------|-------|----------|-------------|-------------|-------|-----------|
| MLACP 2.0           | 0.513 | 0.765    | 0.750       | 0.773       | 0.817 |           |
| MLACP               | 0.256 | 0.677    | 0.283       | 0.911       | 0.744 | 0.000003  |
| mACPpred            | 0.351 | 0.677    | 0.700       | 0.663       | 0.704 | <0.000001 |
| ACPredStackL        | 0.233 | 0.628    | 0.588       | 0.651       | 0.640 | <0.000001 |
is that it cannot predict peptides with more than 50 amino acid residues.

3.5 Model interpretation

MLACP 2.0 was trained using the optimal probabilistic feature vector. As a result, it performed better and more competitively than the previous predictors. The contribution and directionality of the probabilistic features contributed to the meta-model are unknown. Based on a series of recent studies, we have conducted a model interpretation analysis using SHapley Additive explana- tion (SHAP) [48], in order to illustrate the most significant features and their relationship with the outcomes of MLACP 2.0. Fig. 4 shows that MLACP 2.0 generates predictions in the form of line charts above the heatmap matrix ($f(x)$), each feature’s global importance is illustrated in the form of bar graphs on the right-hand side of the heatmap, and the top 20 most important features are listed in order of their global importance. As shown in Fig. 5, we observed that six baseline models based on QSO encoding, and three baseline models based on 10HE, CTDD, seq2vec, and CKSAAGP encoding respectively contributed two baseline models, four encodings (DDE, CTDC, KSC, and AAC) based their respective model contributed the most in the final MLACP 2.0 prediction. The importance of physicochemical properties has been highlighted in previous studies [49,50]. Our analysis also indicates that CKSAAGP is one of the influential features in MLACP 2.0 performance. Among the baseline model comparisons, QSOs had the best performances compared to other encodings (Fig. 2). Therefore, it is not surprising that these models contributed the most to MLACP 2.0. It is interesting to note that the SHAP analysis accurately identifies this phenomenon.

Moreover, r-Distributed Stochastic Neighbor Embedding (r-SNE) was applied to reduce the multidimensional features to two-dimensional plots to understand the relationship between two classes for different encodings. As shown in Fig. 6, QSO and seq2vec encodings (achieved superior performance among baseline models), where ACPs and non-ACPs overlap substantially. It is interesting to note that such overlaps were significantly reduced in the probabilistic features (Fig. 6). The pattern observed in the training was the same on the independent dataset, although non-ACPs are extremely diverse, demonstrating the robustness of our approach.

3.6 Webserver implementation

In order to make the MLACP 2.0 algorithm widely accessible to users, a webserver has been developed, which can be found at https://balalab-skku.org/mlacp2. The web server was built using Django, Python, CSS, HTML, and JavaScript programming languages, as well as a PostgreSQL database for storage and retrieval of job results. Users can find instructions on how to use MLACP 2.0 on the home page and it also includes links to the curated datasets used in the study. The user may upload a file containing multiple FASTA sequences or paste one or more query sequences in FASTA format for prediction. The results of a successful job are displayed in a separate interface, where they can also be downloaded in CSV format for later use. On the submission page, users can view the results of previously completed jobs by entering the job ID into the ‘find job’ option.

![Fig. 4. Comparison of binormal receiver operating characteristics (ROC) curves for ACPs prediction using different methods on an independent dataset.](image)

![Fig. 5. A heatmap plot of the SHAP values for the top 20 probabilistic features based on the training dataset for identifying ACPs.](image)
4. Conclusion

In this study, we developed an improved version of our previous ACP predictor, named MLACP 2.0, based on peptide sequence information. For the development of the second-generation tool, we first constructed non-redundant training and independent datasets based on extensive database and literature searches. It is noteworthy that this is the first study to use such a large non-redundant dataset for modeling or training. Second, 17 different feature encodings and seven different classifiers were employed to develop a pool of baseline models. In the next step, a set of baseline models was manually identified whose predicted ACP values were integrated and trained with CNN to yield the final model.

Several factors contribute to the improved performance of MLACP 2.0, including (i) a reduced training dataset coupled with a meta-model approach; (ii) the predicted probabilistic features have a high intrinsic discriminatory ability on both datasets, resulting in improved performance. Interestingly, this approach can be extended to predict other peptide therapeutic functions [51–54]. Despite its promising performance, MLACP 2.0 also has room for improvement. (i) A novel sequence-based encoding system that is independent of composition and physicochemical properties are expected to be developed and applied in the future. (ii) The use of feature selection techniques [55–59] might help quantify the contribution of each encoding to distinguishing ACPs and non-ACPs. (iii) It may also be possible to develop ensemble deep learning models or hybrid models (conventional and deep learning models) [60] to improve the performance of ACPs when additional datasets become available in the future.

Funding

This work is supported by the National Research Foundation of Korea (NRF) funded by the Korean government (MSIT) (2021R1A2C1014338 and 2021R1C1C1007833), the SungKyunkwan University and the BK21 FOUR (Graduate School Innovation) funded by the Ministry of Education (MOE, Korea) and NRF.

B.M., and J.Y.J, conceived the project and designed the experiments. L.T.P., H.W.P., B.M., T.M., and T.P., performed the experiments and analyzed the data, and B.M. T.P., L.T.P., and H.W.P., wrote the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csbj.2022.07.043.

References

[1] Ortega-Garcia MB, Mesa A, Moya ELJ, Rueda B, Lopez-Ordone G, Garcia JA, et al. Uncovering Tumour Heterogeneity through PKR and nch868 Analysis in Metastatic Colon Cancer Patients Treated with S-FU-Based Chemotherapy. Cancers (Basel) 2020;12.
[2] Bray F, Feletj J, Soejomotaramat I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424.
[3] Palumbo MO, Kavan P, Miller Jr WH, Panasci L, Assouline S, Johnson N, et al. Systemic cancer therapy: achievements and challenges that lie ahead. Front Pharmacol 2013;4:57.
[4] Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: an evolving paradigm. Nat Rev Cancer 2013;13:714–26.
[5] An Z, Flores-Borja F, Irshad S, Deng J, Ng T. Pleiotropic role and bidirectional immunomodulation of innate lymphoid cells in cancer. Front Immunol 2019;10:3111.
[6] Gaspar D, Veiga AS, Castanho MA. From antimicrobial to anticancer peptides: A review. Front Microbiol 2013;4:294.
[7] Morel D, Jeffery D, Aspeslagh S, Almouzni G, Postel-Vinay S. Combining epigenetic drugs with other therapies for solid tumours: past lessons and future promise. Nat Rev Clin Oncol 2020;17:91–107.
[8] Zahreddine H, Borden KL. Mechanisms and insights into drug resistance in cancer. Front Pharmacol 2013;14:4–28.
[9] Raffatellu M. Learning from bacterial competition in the host to develop antimicrobials. Nat Med 2018;24:1097–103.
[10] Xie M, Li M, Yang Y. Anti-cancer peptides: classification, mechanism of action, reconstruction and modification. Open Biol 2020;10:200004.
[11] Shoombuotong W, Schaudiangr N, Nantasenamat C. Unraveling the bioactivity of anticancer peptides as deduced from machine learning. EXCLI J 2018;17:734.
[12] Schweizer F. Cationic amphiphilic peptides with cancer-selective toxicity. Eur J Pharmacol 2009;625:190–4.
[13] Soon TN, Chai AYY, Yap WH, Tang YQ. Anticancer mechanisms of bioactive peptides. Protein Pept Lett 2020;27:823–30.
[14] Fosgerau K, Hoffmann T. Peptide therapeutics: current status and future directions. Drug Discov Today 2015;20:122–8.
[15] Lau JL, Dunn MK. Therapeutic peptides: Historical perspectives, current development trends, and future directions. Bioorg Med Chem 2018;26:2700–7.
[16] Basith S, Manavalan B, Hwan Shin T, Lee G. Machine intelligence in peptide therapeutics: A next-generation tool for rapid disease screening. Med Res Rev 2020;40:1276–314.
[17] Basith S, Manavalan B, Shin TH, Lee DY, Lee G. Evolution of machine learning algorithms in the prediction and design of anticancer peptides. Curr Protein Pept Sci 2020;21:1242–50.
[18] Manavalan B, Basith S, Shin TH, Choi S, Kim MO, Lee G. MALCP: machine-learning-based prediction of anticancer peptides. Oncotarget 2017;8:77121–36.
[19] Fu L, Niu B, Zhu Z, Wu S, Li W. CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics 2012;28:3190–2.
[20] Tyagi A, Tuknait A, Anand P, Gupta S, Sharma M, Mathur D, et al. CancerPPD: a database of anticancer peptides and proteins. Nucleic Acids Res 2015;43: D937–43.
[21] Wang G, Li X, Wang Z. APD3: the antimicrobial peptide database as a tool for research and education. Nucleic Acids Res 2016;44:D1087–93.
[22] Das D, Jaiswal M, Khan FN, Ahamad S, Kumar S. PlantPepDB: A manually curated plant peptide database. Sci Rep 2020;10:2194.
[23] Pirkshalav M, Armstrong AA, Grigolava M, Chubinidze M, Alimbarashvili E, Vishneplesky B, et al. DRAASPV v3: database of antimicrobial/cytotoxic activity and structure of peptides as a resource for development of new therapeutics. Nucleic Acids Res 2021;49:D288–97.
[24] Singh S, Chaudhary K, Dhandha SK, Bhalha S, Usmani SS, Gautam A, et al. SATPdb: a database of structurally annotated therapeutic peptides. Nucleic Acids Res 2016;44:D1119–26.
[25] Fan L, Sun J, Zhou M, Zhou J, Lao X, Zheng H, et al. DRAMP: a comprehensive data repository of antimicrobial peptides. Sci Rep 2016;6:24482.
[26] Shi G, Kang X, Dong F, Liu Y, Zhi N, Hu Y, et al. DRAMP 3.0: an enhanced comprehensive data repository of antimicrobial peptides. Nucleic Acids Res 2022;50:D488–96.
[27] Zhao X, Wu H, Lu H, Li G, Huang Q. LAMP: A Database Linking Antimicrobial Peptides. PLoS ONE 2013;8:e66557.
[28] Quirz C, Saavedra YB, Armijo-Galdames B, Amado-Hinojosa J, Olivera-Nappa A, Sanchez-Daza A, et al. PeptiPedia: a user-friendly web application and a comprehensive database for peptide research supported by Machine Learning approach. Database (Oxford) 2021;2021.
[29] Hsung JH, Chi YH, Li WC, Lin TH, Huang KY, Lee TY. dbAMP: an integrated resource for exploring antimicrobial peptides with functional activities. and physiochemical properties on transcriptome and proteome data. Nucleic Acids Res 2019;47:D285–97.
[30] Chung CR, Kuo TR, Wu LC, Lee TY, Hong JT. Characterization and identification of antimicrobial peptides with different functional activities. Brief Bioinform 2019.
[31] Manavalan B, Patra MC. MLCP: a new computational approach for identifying non-classical secreted proteins based on deep learning. Brief Bioinform 2020;21:1047–57.
[32] Wang X, Li F, Xu J, Rong J, Webb G, Ge Z, et al. AP3BP: a new computational approach for identifying non-classical secreted proteins based on deep learning. Brief Bioinform 2022;23.
[33] Malik A, Subramanyam S, Kim CR, Manavalan B. SortPRed: The first machine learning based predictor to identify bacterial sortases and their classes using sequence-derived information. Comput Struct Biotechnol J 2022;20:165–74.
[34] Li F, Gao X, Xiang D, Pitt ME, Bainomugisa A, Coin LJM. Computational analysis and prediction of PE_PGRS proteins using machine learning. Comput Struct Biotechnol J 2020;22:662–74.
[35] Chai D, Jia C, Zheng J, Zou Q, Li F, Staem5: A novel computational approach for accurate prediction of m5C site. Mol Ther Nucleic Acids Res 2019;47:D285–97.
[36] Wei L, He W, Malik A, Su R, Cui L, Manavalan B. Computational prediction and interpretation of cell-specific replication origin sites from multiple eukaryotes by exploiting stacking framework. Brief Bioinform 2021;22.
[37] Basith S, Hasan MM, Lee C, Wei I, Manavalan B. Integrative machine learning framework for the identification of cell-specific enhancers from the human genome. Brief Bioinform 2021;22.
[38] Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, et al. Scikit-learn: Machine learning in Python. J Mach Learn Res 2011;12:2825–30.
[39] Ke G, Meng Q, Finley T, Wang T, Chen W, Ma W, et al. Lightgbm: A highly efficient gradient boosting decision tree. Adv Neural Inform Process Syst 2017;30:3146–54.
[40] Sharma R, Shirivastava S, Singh SK, Kumar A, Singh AK, Deep-AVPred SS. Artificial intelligence driven discovery of peptide drugs for viral infections. IEEE J Biomed Health Inform 2021.
[41] Sharma R, Shirivastava S, Kumar Singh S, Kumar A, Saxena S, Kumar SR. Deep-AVPred: identifying novel antifungal peptides using pretrained embeddings from seq2vec with 1DCNN-NLSTM. Brief Bioinform 2022;23.
[42] Choller F. Deep learning with Python. Simon and Schuster; 2021.
[43] Hasan MM, Shoombuotong W, Kurata H, Manavalan B. Critical evaluation of web-based DNA N6-methyladenine site prediction tools. Brief Funct Genomics 2021;20:258–72.
[44] Agrawal P, Bhagat D, Mahalwal M, Sharma N, Raghava GPS. AnticP 2.0: an updated model for predicting anticancer peptides. Brief Bioinform 2021;22.
[45] Charoenkwan P, Chiangjong W, Lee VS, Nantasenamat C, Hasan MM, Shoombuotong W. Improved prediction and characterization of anticancer activities of peptides using a novel flexible scoring card method. Sci Rep 2021;11:3682.
[46] Liang X, Li F, Chen J, Li J, Wu H, Li S, et al. Large-scale comparative review and assessment of computational methods for anti-cancer peptide identification. Brief Bioinform 2021;22.
[47] Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 1982;143:29–36.
[48] Stumbelj E, Kononenko I. Explaining prediction models and individual predictions with feature contributions. Knowl Inf Syst 2014;41:647–65.
[49] Chen J, Cheong HH, Liu SW. xDeep-AVPPE: deep learning method for anticancer peptide activity prediction based on convolutional neural network and multitask learning. J Chem Inf Model 2021;61:3789–803.
[50] Yan J, Zhang B, Zhou M, Kwok HW, Sui SW. Multi-Bound-CNN: Classification of ion channel interacting peptides using multi-bound convolutional neural network. Comput Biol Med 2022;147:105717.
[51] Mali R. Learning from bacterial competition in the host to develop antimicrobials. Nat Med 2018;24:1097–103.
[52] Rahman R, Islam M, Islam S. PE_PGRS: a novel computational approach for identifying non-classical secreted proteins based on deep learning. Brief Bioinform 2020;21:1047–57.
[53] Li HL, Pang YH, Liu B. BioSeq-BLM: a platform for analyzing DNA, RNA and protein sequences based on biological language models. Nucleic Acids Res 2021;49:e65.
[54] Chen Z, Zhao P, Li C, Li F, Xiang D, Chen YZ, et al. LearnPlus: a comprehensive and automated machine-learning platform for nucleic acid and protein sequence analysis, prediction and visualization. Nucleic Acids Res 2021;49:e56.
[55] Chen Z, Zhao P, Li F, Marquez-Lago TT, Leier A, Revote J, et al. learn: an integrated platform and meta-learner for feature engineering, machine-learning analysis and modeling of DNA, RNA and protein sequence data. Brief Bioinform 2020;21:1047–57.
[56] Li HL, Pang YH, Liu B. BioSeq-Analysis: a platform for DNA, RNA and protein sequence analysis based on machine learning approaches. Brief Bioinform 2019;20:1280–94.
[57] Liu B, Gao X, Zhang H. BioSeq-Analysis2.0: an updated platform for analyzing DNA, RNA and protein sequences at sequence level and residue level based on machine learning approaches. Nucleic Acids Res 2019;47:e127.
[58] Hasan MM, Tsukiyama S, Cho JY, Kurata H, Alam MA, Liu X, et al. Deepm5C: A deep-learning-based hybrid framework for identifying human RNA N5-methylcytosine sites using a stacking strategy. Mol Ther 2022.