Brain Immunoinformatics

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Breakthrough advances in informatics of the last decade have thoroughly influenced the field of immunology. In particular, the immunoinformatics of the central neural system is referred to as neuroimmunoinformatics (NII). This interdisciplinary overview on NII is addressed to bioscientists and computer scientists. We delineate the dominating trajectories and field-shaping achievements and elaborate on future directions using a bridging language and terminology. Computation, varying from linear modeling to complex deep learning approaches, fuels neuroimmunology through three core directions. Firstly, by providing big-data analysis software for high-throughput methods such as next-generation sequencing and genome-wide association studies. Secondly, by designing models for the prediction of protein morphology, functions, and protein-protein interactions. Finally, NII boosts the output of quantitative pathology by enabling the automatization of tedious processes such as cell counting, tracing, and arbor analysis. Deep sequencing classifies microglia in “sensotypes” to accurately describe the versatility of immune responses to physiological and pathological challenges, as well as to experimental conditions such as xenografting and organoids. NII opts to individualize treatment strategies, personalize disease prognosis and treatment response.

machine learning | brain | neuroimmunology | microglia sensotypes | big data | immunoinformatics

1. Introduction

Breakthrough advances in informatics have thoroughly shaped the field of immunology. The intermingling of machine learning applications with wet lab and clinical results, facilitated by the expanding marketing of user-friendly computer interfaces for lab scientists, has hatched the newly defined society of immunoinformatics. Immunoinformatics flourished in the last decade by detangling tumor immunology, predicting cancer epitopes, and sequencing the adaptive immune receptor repertoires, as has been widely reviewed previously [1][2].

2. Machine Learning for Prediction of Protein, Cellular, and Network Interactions

Studying protein–protein interactions (PPI) is an essential research strategy in molecular biology, including neurobiology. However, screening for PPIs is a time- and resource-consuming process only performed by labs with the required capacity and equipment. Deep learning is a game-changer in PPI research because it allows for the

assembling of molecular databanks, which can be screened for interactive domains in a cost-effective and less time-consuming way [3]. Despite the still low coverage and the low signal-to-noise ratio, computational methods conquer the position of the gold standard in screening, discovering new PPIs, and benchmarking experimental methods.

PPI deep learning methods profit from high-throughput sequencing to reconstruct the primary protein structure. Secondary and tertiary units in possible interaction are predicted based on amino-acid interaction databases [4][5][6][7][8]. Deep learning models, such as the suggested network by Hashemifar et al., predict protein-protein interactions, including homodimerizations, based on the amino-acid sequence alone [3].

2.1. Protein-Epitope Affinity Prediction, Cytokine-Receptor Interactions, and Epitope Prediction

Within the large field of PPIs and sequence-based neuroinformatics, the subfield of protein-epitope interactions is particularly attractive to CNS research. Within this large subcategory, cytokine-receptor interactions (CRI) epitomize a growing field in brain physiology, immunity, and immune-oncology. Numerous examples, such as the role of the CX3CL1-CX3CR1 axis in aging [9][10], synaptic pruning and social behavior [11][12], the role of inflammatory cytokines in depression [13], CCL5-CCR5 in the neurobiology of the glioblastoma multiforme [14], the SDF-1/CXCR4 axis in metastatic brain disease [15], as reviewed by Ransohoff et al. [16][17], should be mentioned. Neural networks can predict CRIs using deep autoencoders; classifiers such as the random forest and K-means have performed satisfactorily in previous research [18]. Deep learning approaches in CRI perpetuate the significance of public repositories. The classifier can be supplied with large databases for high-throughput and data-driven research, which would have been impossible with the available wet lab methods. An indicative list of public repositories that can be engaged in CRI research is provided in **Table 1**.

Besides exploring deep sequencing data for possible PPI/CRI, informatics can provide virtual benchmarks, exploiting the negative sample space in protein interactions. Nath et al. created a database of non-interacting protein pairs, to be used as a gold standard for benchmarking and standardizing the training of CRI-classifiers [19].

Deep learning as a PPI tool is increasingly implemented in epitope prediction after protein folding in secondary and tertiary structures. Liu et al. used deep learning to predict B-cell epitopes and developed a tool (http://ccb1.bmi.ac.cn:81/dlbepitope/) (accessed on 1 July 2021) to be used in adjunction with the **IEDB database** (**Table 1**) [20]. The authors used a four-layered network (**DLBEpitope**) to define the optimal peptide length necessary for accurate epitope prediction of the folded protein, hence offering an epitope-prediction solution and computational economy to the user. Similar developments were noted in the field of epitope-HLA interaction prediction. The SYFPEITHI tool (http://www.syfpeithi.de/bin/MHCServer.dll/EpitopePrediction.htm, accessed on 1 July 2021) predicts the ligation strength of an amino-acid sequence to a defined **HLA type** [21]. Another promising recurrent neural network was published under the abbreviation “MARIA” by Chen et al. MARIA predicts an epitope’s probability to elicit a strong CD4+ T-cell response with an AUC of approximately 0.9 [22]. Similar software

solutions for the prediction of HLA-class-I and HLA-class-II B- and T-cell repertoires are of particular relevance in the field of vaccine design and have been extensively reviewed elsewhere [23][24][25].

2.2. Cell-Cell Interactions and Multiscale Network Modeling

Besides the classical computational neuroscience models integrating pure neuronal circuits [26], machine learning applications are increasingly implemented in studying neuron-glia interactions. Nakae et al. [27] introduce a paradigm of linear modeling to predict neuron-astrocyte interactions using calcium signaling. Transferring this model to the context of NII, a challenging approach would integrate MG to build a three-compartment network model based on the two-compartment Nakae et al. model. Hampered by the lack of excitable membranes [28][29], MG is not accessible by fast signaling methodologies, field-potential electrophysiology, or calcium imaging. While it is known that MG membranes are rich in voltage-, stretch-, or ligand-gated ion channels that tune homeostatic and reactive functions, this type of channel activity is only measurable by single-cell low-throughput methods, such as patch-clamp recording. Therefore, the lack of electrophysiological high-throughput data hampers MG integration into multiscale network modeling [28]. Alternative methodological approaches such as tomato-lectin staining and two-photon in vivo imaging [30][31][32] can infer functional changes and provide material for data-driven hypotheses, especially when combined with deep sequencing methods. Simultaneous gene regulatory network reconstruction from scRNA-seq data such as SCENIC [33] could provide a scaffold for multiscale network models and open new research paths. To our current knowledge, integrating microglial activity in a multimodal gene network context [34] remains an open challenge in neuroimmunoinformatics.

2.3. Probabilistic and Causal Gene Regulatory Networks

Integration of large-scale public datasets with large-scale -omics (e.g., genomic data from whole-genome sequencing and transcriptional data) and implementation of Bayesian statistics can conclude causal inferences on the immunological basis of diseases [35]. As a widely recognized contribution, the association of **TYROBP** as a key driver gene to sporadic Alzheimer's disease was discovered by probabilistic causal network models [36][37]. Despite the broad dynamic of gene regulatory networks in illuminating gene-disease associations, possible pitfalls call for critical interpretation. Regulatory networks are usually cross-sectional studies with a considerable variety of different time points of the disease of interest, which might be a significant confounding factor. Large sample sizes and critical result interpretation should compensate for misconceptions, and cohort studies should opt for different time points of the disease.

3. Machine Learning in Neuropathology and Immunoprofiling

Artificial intelligence applications are gaining popularity in pathology because they bear the attractive potential to overwhelm the rate-limiting step of human processing: the low throughput. Deep learning-based immunopathology takes advantage of high-throughput data from digital pathology [38][39][40][41]. Both supervised and unsupervised machine learning applications are implemented in immunoneuropathology for different purposes [42] and claim improvements in the reading time as well as solutions to subjectivity problems such as the inter-reader variability.

Cell classification from peripheral blood samples is an established computational field with many competing applications. Kutlu et al. compared recurrent convolutional neuronal networks for the white blood cell classification from peripheral blood samples, finding a superior performance of Res-Net [43]. Beyond peripheral blood samples, deep learning algorithms can cope with the complexity of tissues and challenge not only the manual histological semiquantitative imaging, but also older methods of unbiased cell counting [44].

3.1. Microglial Segmentation and Counting

In contrast to peripheral blood cells, MG should be segmented from a noisy background [45]. The highly ramified MG structure [29], described as **lacunarity and fractal dimension** in the technical language of applied mathematics and image analysis, is at the same time a good indicator of the microglial activation status and a technical challenge for computer vision in immunopathology [46][47]. Previous attempts to automatically segment MG have reached a detection accuracy from 80–90%, facing, however, the problem of false negatives due to cell overlapping, texture variabilities, noisy background, and staining inhomogeneities that prohibit the success of standard thresholding models [45][48][49].

Liu et al. circumvented the bottleneck of manual histological image analysis of arborized cells by an unsupervised machine learning pipeline for the high throughput counting of MG and astrocytes [50]. The pipeline improved the analysis time 200 times using an Opera Phenix High Content Screening (PerkinElmer Inc., Hamburg, Germany) high-throughput imaging input. PhenoLOGIC (Phenologic, MI, USA) computer vision is a training image set utilizing supervised machine learning to differentiate background from tissue, integrated by the Harmony High Content Imaging and Analysis software (PerkinElmer), which segmented MG by intensity thresholding. The suggested pipeline for automated image analysis provides the total **Iba1 brain coverage** and extends the analysis to other cellular compartments, such as astrocytes (GFAP and AQP4). Most false-negative results were derived due to inhomogeneities in staining intensity, thus confirming previous observations from independent groups that staining intensity fluctuations are a significant burden for artificial intelligence methods in cell counting [45][49].

Unbiased stereology is an established statistical model for predicting the density of geometrical structures in space (e.g., cells, cellular processes or arbors, synapses, particles) based on randomized cell counting from thick tissue slices. Stereology has contributed to significant advances in the field of neuroimmunology, bridging immune reactions with cognition [51], dementia [52], and epilepsy [53], among others. Mouton et al. [54] performed a longitudinal scientific work towards the automation of the software **Stereologer®** (SRC Biosciences, Tampa FL, USA), and suggested an **Automated Segmentation Algorithm (ASA)** for the deep learning stereology of immunostained neurons and MG in mouse neocortex. ASA is intended to work in a human-in-the-loop interactive pipeline to perform cell segmentation without a priori shape assumptions. Despite the increased shape-complexity of MG, ASA performed a better detection of MG than NeuN-stained neurons [54]. The primary software drawback was the spatial cell overlapping. ASA was subjected to improvements by the same group; Alahmari et al. introduced a next-generation unbiased stereology approach, the **FAST-Stereology (Fully Automated Stereology Technology)**, in a model that improves ASA reading time and reliability for neuronal detection with less than 2% error [55]. The deep learning convolutional neuronal network U-net [56] is trained on a supervised mask for

automated cell counts in the dissector field, thus boosting unbiased stereology with the multiplication power of deep learning. FAST was successfully tested for NeuN measurements in mouse neocortex slices and opened new frontiers for the measurement of more complex and challenging cell appearances, such as densely packed, ramified cells and fragmented branches of astroglia and MG.

Horvath et al. brought about improvements in the detection of ramified cells, influenced by the innovative work of Suleymanova et al. [57]. A deep convolutional neural network approach for astrocyte detection (made available in the software platform FindMyCells©, www.findmycells.org, accessed on 1 July 2021) outperformed classical methods such as ilastik© (https://www.ilastik.org/, accessed on 1 July 2021) and ImageJ© (https://imagej.nih.gov/ij/, accessed on 1 July 2021) in both accuracy and time performance. Compared to manual counts, FindMyCells did not underperform human intelligence in astrocyte counting. Challenging FindMyCells with MG counts and one-by-one by comparing FindMyCells with ASA- or FAST Stereology are open challenges in the field of automated quantitative neuroimmunopathology.

Beyond the field of cell detection and segmentation in statical images, immunoinformatics shapes the field of cell detection and cell tracking in video microscopy. Gregorio da Silva et al. [58] trained a network to detect leukocyte recruitment using **intravital video microscopy** in different contexts, including inflammatory models of the CNS. By setting their available code, the authors provide a valuable immunoinformatic tool to the field of inflammation research in models such as the experimental autoimmune encephalomyelitis [59].

3.2. Cell Arborization Analysis

Dendritic arborization, also known as dendritic branching, is the property of MG, neurons, astrocytes, and other cells, to form new dendritic trees and branches, which anatomically support the establishment of new contact points to their environment. While manual reconstructions of the cell bodies and arbors using standard tools such as Neurolucida® (MBF Bioscience, Williston, VT, USA) can provide clues on the brain immune status [53], they are extremely time-consuming. The urge for an errorless and time-effective automated arborization analysis of cell traces has two constituents: (i) automated cell tracing and (ii) systematic, unbiased quantitative arborization analysis.

Ascoli et al. extensively addressed the problem of a time-effective and objective arborization analysis. Introduced by Scorcioni et al. as the **LM-tool** and improved by Luisi et al. in the **FARSIGHT tool,** this standalone freeware platform offers a multiparametric quantitative arborization analysis using unsupervised co-clustering [60][61]. Lu et al. [62] developed the Scorcioni L-Measure by adding diffusion distance measure and harmonic analysis. Lu et al. identified hierarchical arborization in reconstructed cells and offered a valuable tool for creating hypothesis-free assumptions on microglia morphology and function. Available as the FARSIGHT standalone tool or packaged for MATLAB (MathWorks, Natick, MA, USA), Lu's quantitative analysis is a powerful analysis tool, limited only by the availability of computational resources.

3.3. Automated Cell Arbor Tracing

Automated deep learning-based trace analysis copes with the demand for high-speed processing of reconstructed cells. Even though most automated trace analysis methods were inspired by and dedicated to neuronal arbors, there is an obvious shift towards glia research. MG arborization is associated with functionality. Glia populations are, compared to neurons, more numerous and densely packed, and the deduction of dynamical population trajectories requires the tracing and arborization analysis of large cell datasets, which is hugely time-consuming [53]. This scientific problem drives the design of automated tracing analysis algorithms. Megjhani et al. [63] performed large-scale automated microglial arborization analysis from confocal images. The algorithm is based on a sparse over-complete dictionary learning method and 3D-seeding. The authors feed automated traced cells to a harmonic co-clustering L-measure data analysis pipeline [61] to create cell clusters similar to the benchmark manual reconstructions. Megjhani's results override the accuracy scores of previous methods [64][65], offering a 73% specificity and 95% sensitivity compared to the human benchmark.

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