Computers in Biology and Medicine 104 (2019) 220–226

Contents lists available at ScienceDirect

Computers in Biology and Medicine

journal homepage: www.elsevier.com/locate/compbiomed

Insights into the suitability of utilizing brown rats (*Rattus norvegicus*) as a model for healing spinal cord injury with epidermal growth factor and fibroblast growth factor-II by predicting protein-protein interactions

Nashira Grigg[a], Andrew Schoenrock[b], Kevin Dick[c], James R. Green[c], Ashkan Golshani[a,d], Alex Wong[a], Frank Dehne[c], Eve C. Tsai[e], Kyle K. Biggar[a,d,*]

[a] Department of Biology, Carleton University, Ottawa, Canada
[b] School of Computer Science, Carleton University, Ottawa, Canada
[c] Department of Systems and Computer Engineering, Carleton University, Ottawa, Canada
[d] Department of Biochemistry, Carleton University, Ottawa, Canada
[e] Brain and Mind Research Institute, University of Ottawa, Ottawa, Canada

ARTICLE INFO

Keywords:
Protein interaction
MP-PIPE
EGF
FGF-2
Spinal cord injury

ABSTRACT

The stimulation of the proliferation and differentiation of neural stem cells (NSCs) offers the possibility of a renewable source of replacement cells to treat numerous neurological diseases including spinal cord injury, traumatic brain injury and stroke. Epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF-2) have been used to stimulate NSCs to renew, expand, and produce precursors for neural repair within an adult brown rat (*Rattus norvegicus*). To provide greater insight into the interspecies protein-protein interactions between human FGF-2 and EGF proteins and native *R. norvegicus* proteins, we have utilized the Massively Parallel Protein-Protein Interaction Prediction Engine (MP-PIPE) in an attempt to computationally shed light on the pathways potentially driving neurosphere proliferation. This study determined similar and differing protein interaction pathways between the two growth factors and the proteins in *R. norvegicus* compared with the proteins in *H. sapiens*. The protein-protein interactions predicted that EGF and FGF-2 may behave differently in rats than in humans. The identification and improved understanding of these differences may help to improve the clinical translation of NSC therapies from rats to humans.

1. Introduction

Neurological diseases, disorders, and injuries represent one of the leading causes of disability as very few neurological conditions are curable, and many degrade over time [1]. Over the past 25 years, the burden of neurological disorders has increased substantially worldwide due to expanding population numbers and aging, despite substantial decreases in mortality rates from stroke and communicable neurological disorders [2].

The repair of neurological diseases such as stroke, traumatic brain injury or spinal cord injury may be addressed through the use of dormant neural stem cells– undifferentiated, self-renewing cells within the adult brain and spinal cord that can give rise to a multitude of daughter cell types. Recent studies have attempted to induce endogenous repair by inducing the pre-existing neural stem cells via the administration of proteins to the brain or spinal cord. Specifically, the addition of two growth factors, human epidermal growth factor (i.e., EGF), and human fibroblast growth factor 2 (i.e., FGF-2). These growth factors have shown to induce proliferation of spinal cord ependymal precursor cells both *in vivo* and *in vitro* [3].

The combination of EGF and FGF2 has been demonstrated to be a promising new therapy for spinal cord injury and can be utilized to restore cognitive function in an animal with brain and stroke injury [4]. In 2017, Lou et al. successfully utilized EGF and FGF-2 to repair traumatic tympanic membrane perforations in human ears, which resulted in significantly accelerated healing times. The growth factors were applied directly inside the ears of the human subjects, inducing the proliferation of fibroblasts and epithelial cells, increasing re-vascularization which overall reduced the time for the perforations within the tympanic membrane to close [5]. Fibroblast growth factors 1 and 2 have been found to be involved in angiogenesis [6]. Research has determined that the unregulated expression of these proteins will also

* Corresponding author. Department of Biology, Carleton University, Ottawa, Canada.
E-mail address: kyle_biggar@carleton.ca (K.K. Biggar).

https://doi.org/10.1016/j.compbiomed.2018.11.026
Received 7 September 2018; Received in revised form 28 November 2018; Accepted 29 November 2018

cause uncontrolled proliferation [7,8]. Additionally, EGF has been found to be expressed during the adult and developing stages of the central nervous system. The protein has also been demonstrated to promote the growth of neurites, the projection from neurons [9,10]. There is no evidence of the two proteins to interact with each other directly, however, research has emerged suggesting that the two interact with similar partners [11]. This may convey that the use of both proteins may be vital to their function in inducing proliferation within stem cells. Both EGF and FGF-2 interact with the cell in an extracellular capacity.

Currently, the common animal model for studying the effect of EGF and FGF-2 on SCI in humans is the Sprague-Dawley rat (*Rattus norvegicus*) [12,13]. Interestingly, it is the human EGF and FGF-2 proteins that are utilized within rat cell cultures, as well as the *in vivo* studies on rats to determine their effect on SCI repair. The conclusions of these studies are applied to technologies for use in humans, however, there are likely direct implications in cross-species protein interactions arising from species-specific protein sequence changes that have been primarily overlooked to date.

To provide insights into potential cross-species protein-protein interactions (i.e., PPIs) that could be at play in this SCI model, we employed the MP-PIPE program, an algorithm that predicts novel interactions through the study of previously known PPIs. The algorithm allows for a vast number of proteins pairs to be analyzed in parallel to determine all possible PPIs with a high degree of accuracy [14]. Cellular PPI analysis completed in the wet-lab are typically subject to a very high cost both in time and funding, along with many technical limitations and false positives. To aid in the validation of *in silico* methods to provide insight into *in vivo* protein interactions, one previous study tested the overall accuracy of the MP-PIPE in predicting protein interaction in yeast [15]. This study used a simplified dataset for validation and demonstrated a 61% sensitivity (the proportion of true positive values in regards to the sum of true positives and false negatives) for detecting PPIs predicted by MP-PIPE with 89% specificity (the proportion of true negative values versus the added total value of false positives and true negatives) and 75% accuracy [15]. Interestingly, these results that are similar to those of common analytical biochemical techniques and highlight the utility of *in silico* approaches to uncovering novel protein interactions. Furthermore, an independent comparison of PIPE to three other computational prediction methods [16–18] determined that MP-PIPE outperformed all other methods in high specificity PPI prediction [19]. Another study completed in 2014 utilized MP-PIPE to predict all high probability PPIs within the human proteome [20]. More recently, the MP-PIPE algorithm has also been used in a cross-species manner to predict PPIs between the Zika virus and humans [21]. Given that MP-PIPE has shown the ability to predict PPIs with high confidence in a cross-species context [14,15,20,22–24], we utilized MP-PIPE to determine the interactions between short co-occurring polypeptide sequences within EGF and FGF-2. The designated protein sequences were further run against the human proteome to determine possible interactions and interferences that would affect the protein's function.

This study investigates the hypothesis that FGF-2 and EGF proteins are viable to induce the proliferation of human neurospheres and may be a possible therapeutic for SCI. As rats are often the typical model organism to study the induced proliferation of stem cells to heal injuries, this study compares the PPIs within human and rat proteomes. MP-PIPE was applied to the human and rat proteome to determine PPIs with human growth factors EGF and FGF-2, as these human proteins are commonly utilized in rat studies.

2. Materials and methods

2.1. Database creation

MP-PIPE requires the creation of an indexed database of protein sequences and known interactions before any predictions may be created. Separate databases were created for human and rat cells, including all known proteins and their interactions. To construct the databases for this project, POSITOME was run on both *H. sapiens* and *R. norvegicus* separately, with the same parameters [25]. POSITOME is a web service that collects all the information on the organism of interest from open access protein databases that include links to all papers published on said interactions. Conservative settings were applied, meaning only physical protein-protein interactions detected by high accuracy methods such as two-hybrid, affinity capture, reconstituted complex, and co-localization are included. POSITOME produces two output files, one containing the protein pairs, the other with the protein sequences. Finally, the two-human growth factor protein sequences for FGF-2 and EGF were added at the end of the rat and human protein sequence files unless they were already present in the file, as they were with the human file.

The resulting files for the *H. sapiens* database included 11,787 proteins and 63,747 known pairs. As for the *R. norvegicus* files, only 1051 protein sequences and 1156 known protein pairs were included.

2.2. Setup script run

Once the database had been created, a setup script was run to render the protein pairs into a format more efficient for PIPE. The setup script first reviews the input files to ensure they are formatted properly to ensure no faulty sequences had been entered. The rest of the script formats files for the PIPE run, constructing a database file with all proteins, and index files that allow PIPE to run more efficiently.

2.3. PIPE runs

An overview of our experimental methods can be seen in Fig. 1. After the database had been created, an interaction graph was established. In this graph, every protein is represented as a vertex, and the interactions between two separate proteins are erected as edges. Essentially, this graph acts as an indicator of all known interactions between proteins determined in the database creation step.

PIPE will make use of this data to predict if two query proteins will interact. We may assume we would like to predict whether EGF interacts with some other protein deemed X as an example. The PIPE run begins with a sliding window that is run over the EGF protein sequence, generating fragments of 20 amino acids in length (this window size may be altered within the MP-PIPE script). This EGF fragment is compared to fragments of the same size made up of the other proteins in the database. The fragments are lined up with one another and each amino acid pair is then compared with a substitution matrix where the columns and rows represent values indicative of the probability that one of the 20 essential amino acids may be replaced by the other through evolution. PIPE utilizes the PAM1 matrix raised to the power of 120, or the PAM^{120} matrix. If the matrix value is larger than a threshold value set to 35, it is deemed as a match. If the same fragment pair reports over than 10 matches for each other, then the fragments are deemed similar. These threshold values were determined through previous experimental validation [14], a process which required roughly 400 h to determine that these values were the most selective in the evaluation of protein fragment interaction. When a protein has a fragment similar in sequence to the EGF fragment, all this new protein's neighbours in the interaction graph are added to a list.

Next, a protein X (the second query protein) is run with the same sliding window method over all the proteins in the list generated. If there is similarity between two fragments this time, this means that proteins with similar regions to EGF and protein X are known to interact and acts as evidence that out query proteins potentially interact, and a value in a result matrix is incremented for the two fragments. After a result matrix is computed for EGF and protein X, a simplified median filter is applied. This filter looks at the neighbours of a query

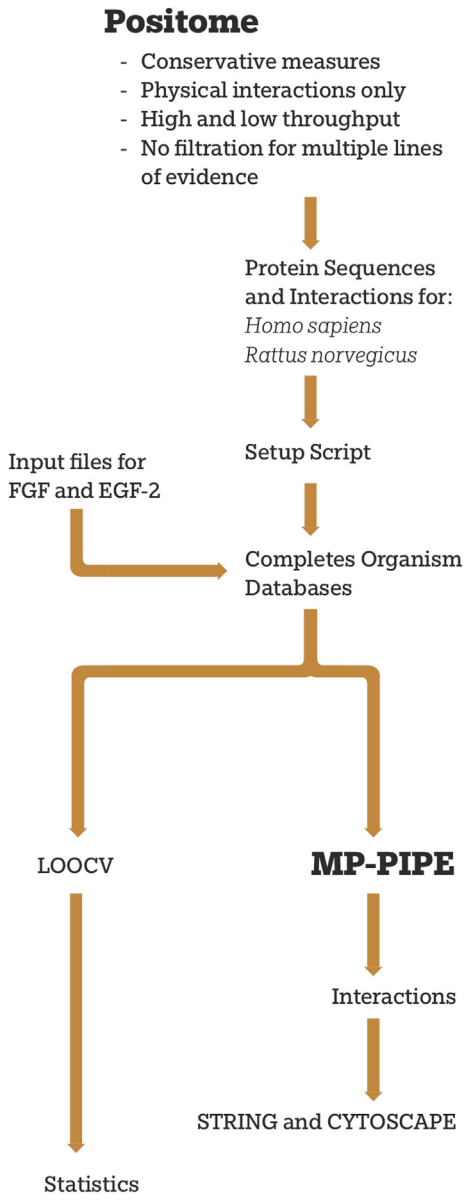

Fig. 1. Overview of methods used to predict novel EGF and FGF-2 protein interactions using MP-PIPE.

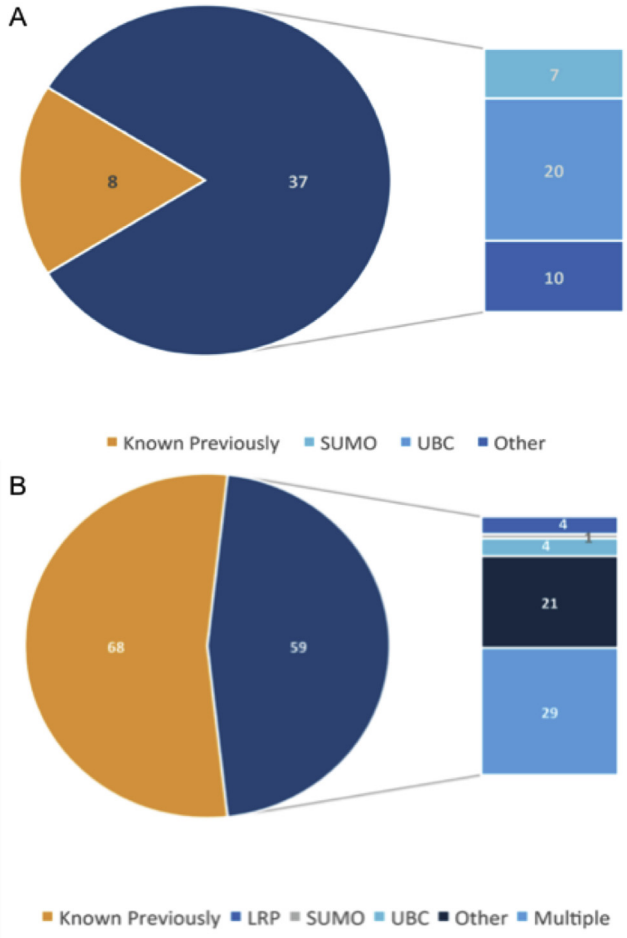

Fig. 2. Summary of predicted protein interactions. Cellular processes that are enriched from either (A) rat or (B) human proteins predicted by MP-PIPE to interact with human FGF-2 and EGF growth factors. SUMO represents the family of small ubiquitin-like modifier (SUMO) proteins and the polyubiquitin-c precursor (UBC) protein family, and lipoprotein receptor-related (LRP) proteins.

cell, if the neighbours are mostly zeros meaning no similarity between fragments, then the query cell would also be set to zero. If this were not the case, the query cell is set to one. The values of the cells of the result matrix are averaged, giving the PIPE score which indicates how likely it is that the proteins interact [14].

2.4. LOOCV run

Leave-one-out cross-validation or LOOCV is a statistical test done to determine the efficiency of a prediction. Essentially, each known PPI is removed from the database, one at a time, to see if PIPE can predict an interaction between these proteins known to interact using the other available data. Based on these results, MP-PIPE's performance can be evaluated and appropriate score thresholds (used to determine if a given query pair is predicted to interact) can be established.

Once the LOOCV test was completed, an effective cut off was generated for the PIPE scores. In the case of *R. norvegicus*, cut-off values were assessed at more lenient levels due to the reduced amount of protein sequences and interactions present in the database. The cut off was set such that MP-PIPE achieved a sensitivity of 90%. In the case of *H. sapiens*, the database yielded more proteins sequences and interactions, so the cut off was set such that MP-PIPE achieved a sensitivity of 99%.

After the LOOCV was applied to the PIPE results, the list of proteins interacting with EGF and FGF-2 were represented by UniProt ID. Each ID was entered into the UniProt database, so that the common protein name, interactions, and basic functions could be determined. In addition to UniProt, BioGRID 3.4 was also utilized to determine if the interactions had been mentioned in a previous study.

2.5. Visualization of enriched protein interaction networks

To characterize the function and potential interplay between protein-protein interactions were mapped using the STRING database and this was subsequently modeled using CYTOSCAPE software and clustered using MCL clustering based on STRING combined scores. Pathway enrichment analysis for protein interaction clusters was performed on proteins predicted to have significant interaction by MP-PIPE (Fig. 1). This analysis was performed using the Gene Ontology (GO) annotations available for *H. sapiens* using the PANTHER classification system with default settings (v.11.1).

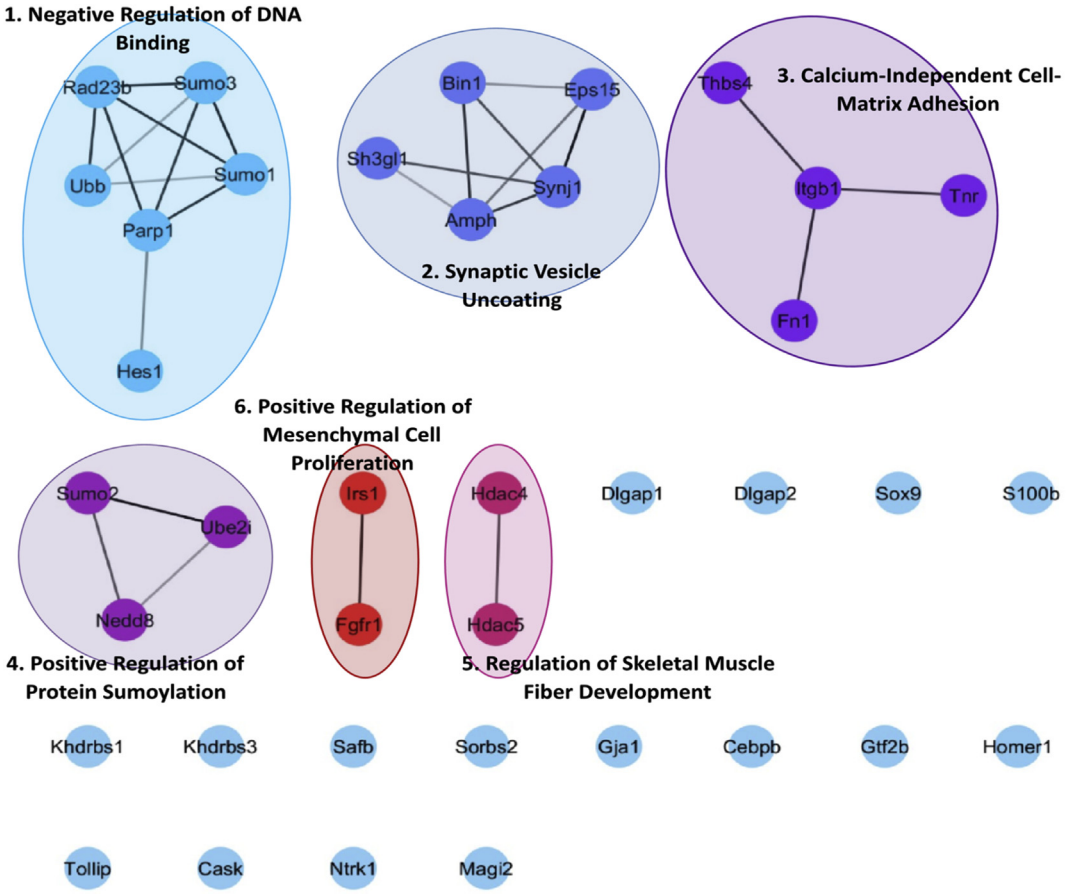

Fig. 3. The protein mapping results for the proteins within rat cells that are predicted to interact with EGF and FGF-2, sorted by gene function within the cell.

3. Results and discussion

MP-PIPE required approximately 6 h to predict the interactions of FGF-2 and EGF with the protein database for *H. sapiens*. After LOOCV, 59 novel protein interactions for both growth factors were predicted by MP-PIPE, with 39 for EGF and 20 for FGF-2; 68 additional interactions that had been previously identified in the literature were determined, including 30 for EGF and 38 for FGF-2. For *R. norvegicus*, MP-PIPE took approximately 3 h to run through the interactions for the two human growth factor proteins. Once LOOCV had been applied, 37 novel interactions were discovered, showing 14 for EGF and 23 for FGF-2 and 8 previously known interactions were presented with 5 for EGF and 3 for FGF-2. The proteome for *R. norvegicus* within the database was approximately ten times smaller than that for *H. sapiens*, resulting in a correspondingly lower number of predicted interactions. All interaction data are listed in Supplemental Table S1.

Of the unique predicted interactions with the growth factors among human proteins, many interactions were enriched in cellular processes that involve post-translational modifications such as the small ubiquitin-related modifier (SUMO) family, the cullin (CUL) family, and the polyubiquitin-c (UBC) family. There was also involvement in the low-density lipoprotein (LDL) receptor family, matrilins, zinc finger family, and the NEDD4 sodium channel protein family. As for the novel predictions by MP-PIPE within the rat proteome, UBC and SUMO processes were also identified (Fig. 2).

Considering the novel human interactions predicted by PIPE, 29 of the interacting proteins are involved with the LDL receptor and lipoprotein receptor-related proteins (LRPs), as shown in Fig. 2A. The LDL family of proteins is involved in receptor-mediated endocytosis, or the transport of molecules within the cell. EGF precursors have been shown to exist in the family of LRPs [26]. Also, LRPs have been found to interact with EGF receptors. Thus, EGF and LRPs may well interact with each other's receptors. It has also been suggested that LRP may affect the activity of astrocytes in neuroplasticity [27].

The SUMO protein family is involved with cellular processes such as transport, transcription, chromosome movement, and DNA repair. The SUMO family performs post-translational modifications (PTMs) of proteins [28]. It should be noted that most of the human proteins predicted to interact with the SUMO group are also found to interact with other protein families such as NEDD, CUL, and LRP. In contrast, the PPI predictions for the growth factors and proteins within rat cells are predicted to interact with proteins related to SUMOs which specifically induce transcription, differing from the PPIs predicted for human cells. There has been a suggestion of a relationship between FGF signaling and the modification by SUMO in human embryonic development [29], but little has been shown that these two interact directly or through another protein. Recent studies have involved the fusion of SUMO genes to those transcribing EGF to aid in the expression of the EGF protein *in vivo* [30].

The UBC and its related proteins are another family involved in PTMs, typically acting to tag a protein for proteasomal degradation. It should be noted that 5/6 of the predicted protein interactors within the human proteome are not human proteins, but are proteins from either rat or mouse proteomes. This type of crossover has been seen previously in the databases utilized in this experiment and are most likely due to transgenic experiments which do not represent *in vivo* interactions. The one novel interaction between EGF and a human protein is ubiquitin carboxyl-terminal hydrolase 3, a protein known to deubiquitinate proteins that have been monoubiquitinated, typically histones [31]. EGF has been shown to interact with E3 ubiquitin-protein ligase [32], also seen in the human interactions that PIPE predicted that were previously known, although the ligase ubiquitinates proteins to mark

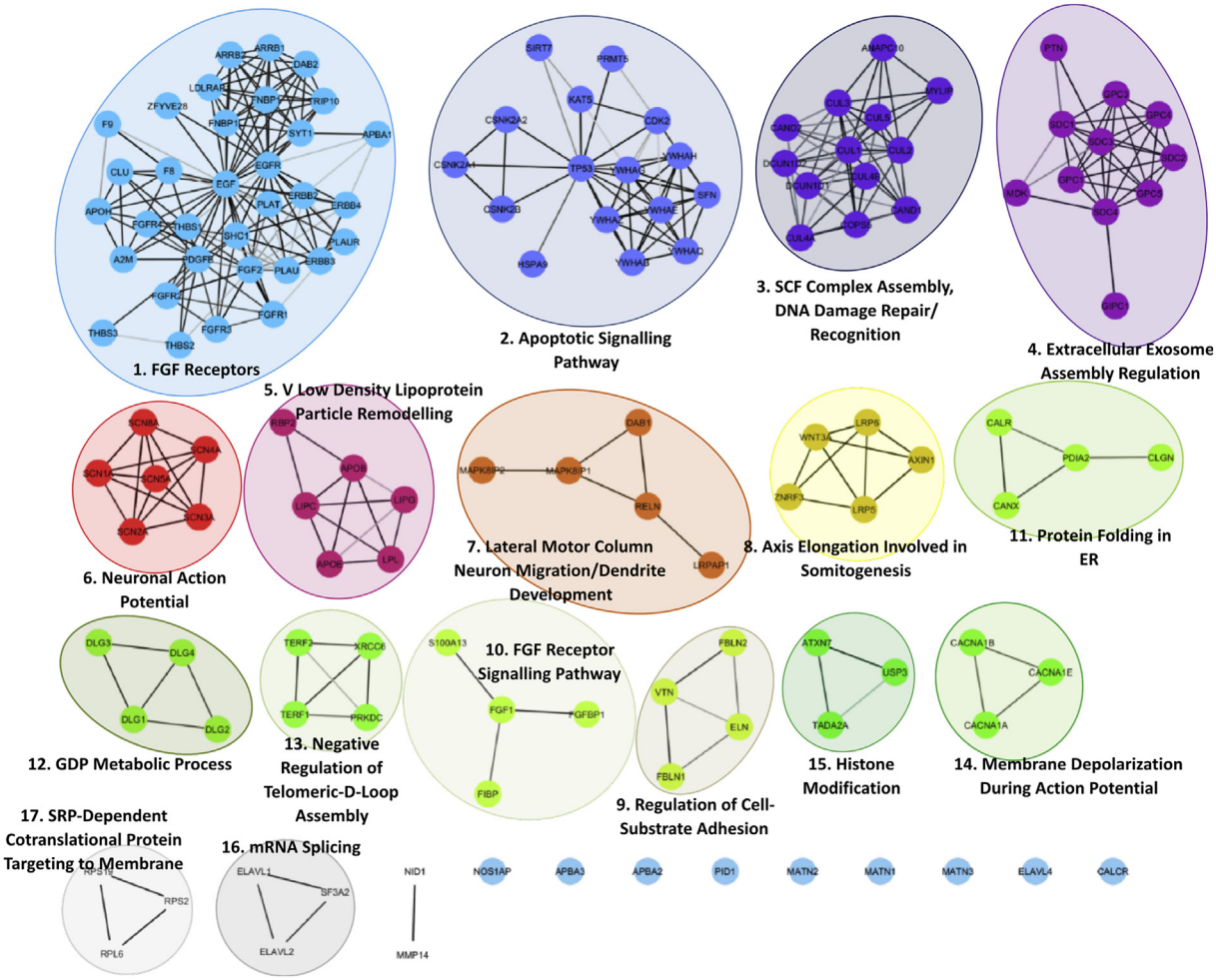

Fig. 4. The highly scored human proteins predicted to interact with growth factors EGF and FGF-2, mapped to display their family and function within the human cell.

Table 1
MP-PIPE predicted PPIs with EGF and FGF-2 for the human proteome confirmed by the STRING predictive database.

EGF or FGF-2	Uniprot ID of STRING Predicted Interactor	Name of STRING Predicted Interactor	STRING Method of Prediction
EGF	P10523	S-antigen	Textmining
EGF	Q5T0N5	Formin binding protein 1-like	Textmining, Curated Databases
EGF	Q15642	Thyroid hormone receptor interactor 10	Textmining, Curated Databases
FGF-2	P35442	Thrombospondin 2	Textmining
FGF-2	P35052	Glypican 1	Textmining, Curated Databases, Co-Expression

Table 2
MP-PIPE predicted PPIs with FGF-2 for the rat proteome confirmed by the STRING predictive database.

Uniprot ID of STRING Predicted Interactor	Name of STRING Predicted Interactor	STRING Method of Prediction
F1LYL9	Sox9	Textmining
P49134	Integrin beta-1	Textmining, Curated Databases

them for degradation. Within the results of the rat proteome with human EGF and FGF-2, there were no cross-species predictions, and more PPIs involving UBC were present. This suggests that FGF-2 and EGF may have a different role in rat cells than in human cells, one that is more involved in regulating ubiquitination or it may be due to the limited rat training data set producing very few novel interactions outside of the known local neighbourhood. The related functions to the involved proteins are listed in Fig. 3. Within human cells EGF shows involvement in the matrilin family, with one predicted interactor showing a known interaction with matrilin-4 and the cartilage matrix protein, also known as matrilin-1. The members of the matrilin family share similar structure, including an epidermal growth factor-like domain. Members of the matrilin family have been shown to interact with themselves, which might explain the predicted interactions [33]. PIPE also predicted EGF to interact with proteins involved in the NEDD4 family, specifically with the sodium channel proteins within human cells. EGF has been known to upregulate ion channel proteins within neurons and muscles which has been linked to metastatic behaviours

within cells [34]. EGF has been found to cause cancer if not regulated properly within cells, suggesting a safe dosage should be determined [35].

Both FGF-2 and EGF have been predicted to interact with proteins involved with the Cullins, a family involved in PTMs with ubiquitin for protein degradation [36]. This can be seen in the human result protein map in Fig. 4. Recent studies have shown that the EGF receptor will phosphorylate the proliferating cell nuclear antigen, a key component of DNA synthesis when the cell is stimulated to grow. The phosphorylation of this protein by the EGF receptor essentially protects it from being degraded, so that the cell can continue to grow. The proliferating cell nuclear antigen is susceptible to ubiquitination by the Cullin-4A protein, but this can also be stopped with the EGF receptor [37].

Further validation of the PPI predictions presented was performed with use of the STRING predictive database. Utilizing the list of novel PPI predictions produced by MP-PIPE, STRING predicted interactions within the human proteome between EGF and three proteins as well as FGF-2 with two proteins, as shown in Table 1. With regards to the rat proteome, Table 2 lists the STRING predicted novel PPIs also predicted by MP-PIPE for FGF-2 with two proteins however none were confirmed for EGF.

This study utilized MP-PIPE to determine interactions between EGF and FGF-2 with both Rat and Human proteomes. Here PPIs were successfully predicted in both human and rat cells with the growth factors, as many of the predictions had been reported-an indication of the accuracy of the software. The PPIs predicted may suggest that EGF and FGF-2 may behave differently in rats than in humans, however the use of PIPE on the rat proteome is not as accurate as it is on humans as the protein database is much smaller comparably. Additional experimental analysis is required to validate the novel PPIs that have been presented in this study. For example, the enriched interaction of human EGF and FGF-2 with the SUMO protein family in rats. Overall, we present the ability to utilize predictive tools such as MP-PIPE to determine a model organism with ideal proteomic similarity to better model the EGF and FGF-II pathway within human cells for spinal cord repair. It is important to understand the similarities and differences between the animal and human models in order to more successfully translate promising animal therapies to humans.

Conflicts of interest

None declared.

Acknowledgements

This research was funded by an NSERC discovery grant to KK Biggar.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.compbiomed.2018.11.026.

List of abbreviations

SCI	Spinal cord injury
FGF-2	Basic fibroblast growth factor
EGF	Pro-epidermal growth factor
MP-PIPE	The Massively Parallel Protein-Protein Interaction Prediction Engine
PPIs	Protein-protein interactions
LDL receptor	Low-density lipoprotein receptor
LRPs	LDL receptor related proteins
SUMO	Small ubiquitin-related modifier
PTMs	Post translational modification
UBC	Polyubiquitin-C

References

[1] Canadian Institute for Health Information, The Burden of Neurological Diseases, Disorders and Injuries in Canada, CIHI, 2007.
[2] Valery L. Feigin, et al., Global, regional, and national burden of neurological disorders during 1990-2015: a systematic analysis for the Global Burden of Disease Study 2105, Lancet Neurol. 16 (11) (2015) 877–897.
[3] N. Silva, N. Sousa, R. Reis, A. Salgado, From basics to clinical: a comprehensive review on spinal cord injury, Prog. Neurobiol. 114 (2014) 25–57.
[4] B. Kolb, C. Morshead, C. Gonzalez, M. Kim, C. Gregg, T. Shingo, S. Weiss, Growth factor-stimulated generation of new cortical tissue and functional recovery after stroke damage to the motor cortex of rats, J. Cerebr. Blood Flow Metabol. 27 (5) (2007) 983–997.
[5] Z. Lou, Z. Lou, A comparative study to evaluate the efficacy of EGF, FGF-2, and 0.3% (w/v) ofloxacin drops on eardrum regeneration, Medicine (Baltim.) 96 (30) (2017) e7654.
[6] Y. Shimoyama, M. Gotoh, Y. Ino, M. Sakamoto, K. Kato, S. Setsuo, Characterization of high-molecular-mass forms of basic fibroblast growth factor produced by hepatocellular carcinoma cells: possible involvement of basic fibroblast growth factor in hepatocarcinogenesis, Jpn. J. Canc. Res. 82 (11) (1991) 1263–1270.
[7] X. Zhang, O. Ibrahimi, S. Olsen, H. Umemori, M. Mohammadi, D. Ornitz, Receptor specificity of the fibroblast growth factor family, J. Biol. Chem. 281 (23) (2006) 15694–15700.
[8] M. Okada-Ban, J. Thiery, J. Jouanneau, Fibroblast growth factor-2, Int. J. Biochem. Cell Biol. 32 (3) (2000) 263–267.
[9] P. Hermann, R. Van Kesteren, W. Wildering, S. Painter, J. Reno, J. Smith, et al., Neurotrophic actions of a novel Molluscan epidermal growth factor, J. Neurosci. 20 (17) (2000) 6355–6364.
[10] H. Lu, J. Chai, M. Li, B. Huang, C. He, R. Bi, Crystal structure of human epidermal growth factor and its dimerization, J. Biol. Chem. 276 (37) (2001) 34913–34917.
[11] T. Chen, K. Lin, C. Chen, S. Lee, P. Lee, Y. Liu, et al., Using an in situ proximity ligation assay to systematically profile endogenous protein–protein interactions in a pathway network, J. Proteome Res. 13 (12) (2014) 5339–5346.
[12] H. Mestre, M. Ramirez, E. Garcia, S. Martiñón, Y. Cruz, M. Campos, et al., Lewis, fischer 344, and sprague-dawley rats display differences in lipid peroxidation, motor recovery, and rubrospinal tract preservation after spinal cord injury, Front. Neurol. 6 (2015).
[13] P. Popovich, P. Wei, B. Stokes, Cellular inflammatory response after spinal cord injury in sprague-dawley and lewis rats, J. Comp. Neurol. 377 (3) (1997) 443–464.
[14] S. Pitre, F. Dehne, A. Chan, J. Cheetham, A. Duong, A. Emili, et al., PIPE: a protein-protein interaction prediction engine based on the re-occurring short polypeptide sequences between known interacting protein pairs, BMC Bioinf. 7 (1) (2006) 365.
[15] S. Pitre, C. North, M. Alamgir, M. Jessulat, A. Chan, X. Luo, et al., Global investigation of protein-protein interactions in yeast saccharomyces cerevisiae using re-occurring short polypeptide sequences, Nucleic Acids Res. 36 (2008) 4286–4294.
[16] Y. Guo, L. Yu, Z. Wen, M. Li, Using support vector machine combined with auto covariance to predict protein-protein interactions from protein sequences, Nucleic Acids Res. 36 (9) (2008) 3025–3030.
[17] S. Martin, D. Roe, J.L. Faulon, Predicting protein-protein interactions using signature products, Bioinformatics 21 (2) (2005) 218–226.
[18] J. Shen, J. Zhang, X. Luo, W. Zhu, K. Yu, K. Chen, et al., Predicting protein-protein interactions based only on sequences information, Proc Nat Acad Sci USA 104 (11) (2007) 4337–4341.
[19] Y. Park, Critical assessment of sequence-based protein-protein interaction prediction methods that do not require homologous protein sequences, BMC Bioinf. 10 (2009) 419.
[20] A. Schoenrock, B. Samanfar, S. Pitre, M. Hooshyar, K. Jin, C. Phillips, et al., Efficient prediction of human protein-protein interactions at a global scale, BMC Bioinf. 15 (1) (2014).
[21] T. Kazmirchuk, K. Dick, D. Burnside, B. Barnes, H. Moteshareie, M. Hajikarimlou, et al., Designing anti-Zika virus peptides derived from predicted human-Zika virus protein-protein interactions, Comput. Biol. Chem. 71 (2017) 180–187.
[22] K. Omidi, M. Hooshyar, M. Jessulat, B. Samanfar, M. Sanders, D. Burnside, et al., Phosphatase Complex Pph3/Psy2 Is Involved in Regulation of Efficient Non-Homologous End-Joining Pathway in the Yeast Saccharomyces cerevisiae, PloS One 9 (1) (2014) e87248.
[23] B. Samanfar, S.J. Molnar, M. Charette, A. Schoenrock, F. Dehne, A. Golshani, et al., Mapping and identification of a potential candidate gene for a novel maturity locus, E10, in soybean, Theor. Appl. Genet. 130 (2) (2017) 377–390.
[24] K. Omidi, M. Jessulat, M. Hooshyar, D. Burnside, A. Schoenrock, T. Kazmirchuk, et al., Uncharacterized ORF HUR1 influences the efficiency of non-homologous end-joining repair in Saccharomyces cerevisiae, Gene 639 (2018) 128–136.
[25] K. Dick, F. Dehne, A. Golshani, J.R. Green, Positome: a method for improving protein-protein interaction quality and prediction accuracy, IEEE CIBCB (2017) 1–8.
[26] H. Gu, A. Adijiang, M. Mah, D. Zhang, Characterization of the role of EGF-A of low density lipoprotein receptor in PCSK9 binding, JLR (J. Lipid Res.) 54 (12) (2013) 3345–3357.
[27] I. Hussaini, M. Brown, L. Karns, J. Carpenter, G. Redpath, S. Gonias, et al., Epidermal growth factor differentially regulates low density lipoprotein receptor–related protein gene expression in neoplastic and fetal human astrocytes, Glia 25 (1) (1999) 71–84.
[28] J. Gareau, C. Lima, The SUMO pathway: emerging mechanisms that shape specificity, conjugation and recognition, Nat. Rev. Mol. Cell Biol. 11 (12) (2010) 861–871.

[29] E. Pauws, P. Stanier, FGF signalling and SUMO modification: new players in the aetiology of cleft lip and/or palate, Trends Genet. 23 (12) (2007) 631–640.
[30] Z. Su, Y. Huang, Q. Zhou, Z. Wu, X. Wu, Q. Zheng, et al., High-level expression and purification of human epidermal growth factor with SUMO fusion in Escherichia coli, Protein Pept. Lett. 13 (8) (2006) 785–792.
[31] F. Nicassio, N. Corrado, J. Vissers, L. Areces, S. Bergink, J. Marteijn, et al., Human USP3 is a chromatin modifier required for S phase progression and genome stability, Curr. Biol. 17 (22) (2007) 1972–1977.
[32] C. Hong, S. Duit, P. Jalonen, R. Out, L. Scheer, V. Sorrentino, et al., The E3 ubiquitin ligase IDOL induces the degradation of the low density lipoprotein receptor family members VLDLR and ApoER2, J. Biol. Chem. 285 (26) (2010) 19720–19726.
[33] D. Piecha, C. Wiberg, M. Mörgelin, D.P. Reinhardt, F. Deák, P. Maurer, et al., Matrilin-2 interacts with itself and with other extracellular matrix proteins, Biochem. J. 367 (3) (2002) 715–721.
[34] F. Deák, R. Wagener, I. Kiss, M. Paulsson, The matrilins: a novel family of oligomeric extracellular matrix proteins, Matrix Biol. 18 (1) (1999) 55–64.
[35] S. Fraser, I. Ozerlat-Gunduz, W. Brackenbury, E. Fitzgerald, T. Campbell, R. Coombes, et al., Regulation of voltage-gated sodium channel expression in cancer: hormones, growth factors and auto-regulation, Philos. Trans. R. Soc. Lond. B Biol. Sci. 369 (1638) (2014) 20130105.
[36] Y. Lo, P. Ho, S. Wang, Epidermal growth factor receptor protects proliferating cell nuclear antigen from cullin 4A protein-mediated proteolysis, J. Biol. Chem. 287 (32) (2012) 27148–27157.
[37] R. Bodnar, Epidermal growth factor and epidermal growth factor receptor: the Yin and Yang in the treatment of cutaneous wounds and cancer, Adv. Wound Care 2 (1) (2013) 24–29.