

Chapter 2

Coralyne Targets Proteases Involved in Cancer Progression: An In Silico Study

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Abstract Molecular docking has a significant application in finding the targets involved in cancer meta stasis. A positive correlation between the vigourness of tumor and expression of various proteases has been established, which includes serine proteases like furin and uPA, matrix metalloproteinases such as membrane type 1 MMP and tissue inhibitor of metalloproteinases, cysteine proteases such as cathepsin B & S and aspartate protease like cathepsin D. Virtual screening based on structure and post-screening analysis are routinely used in search of novel lead compound and its optimization. In the present study, the binding energy of coralyn with various metastatic proteases was analyzed using in silico docking tools such as iGEMDOCK v2.1, hex v6.3 and patch dock. The analysis of results indicates that coralyn exhibited significantly good binding affinity with furin and uPA predicting the possibility of coralyn in regulating cancer invasion and metastasis. Further, protein-protein network was analyzed using STRING version 10 based on KEGG pathways and clustered into groups using on MCL and k-mean algorithms to unfold its interacting partners proteins in cancer metastasis.

Keywords Coralyn · Metastatic proteases · iGEMDOCK · Hex · STRING

2.1 Introduction

Cancer is an unchecked growth of cells, which turns into serious disease conditions. Thus, development of a new drug against cancer is a vital step. Proteolysis is one of the core biological event involved in metastasis, cell proliferation, angiogenesis and apoptosis. Serine proteases, the largest human protease gene family, mediate a multifariousness events relevant to metastasis. Cancer invasion and metastasis are a result of degradation of basement membrane mainly by metallo-

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proteinases (MMPs) [1]. Mammalian cysteine proteases are confined to lysosome such as cathepsins B, S, H, and L or in the cytosol like calpains. Reports have suggested that there is an interrelationship between the activity of cysteine proteases and aggressiveness of cancer as they can degrade both intracellular and extracellular matrix (ECM) proteins. Cathepsin B is involved in the disintegration of connective tissue and basement membrane which lead to cancer metastasis [2]. Aspartic proteases are enzymes which contain two lobes bridged by a cleft containing the catalytic site with two aspartate residues. Cathepsin-D is a pervasive aspartic endoprotease distributed in lysosomes. Studies suggest that variation in expression of cath-D may play an important role in cancer metastasis by favoring the growth of micro-metastases [3]. Furin is an important member of the family of pro-protein processing enzyme and highly expressed in a variety of tumors. Many proteins which are closely related to tumor development, including Notch, Wnt, MT1-MMP and VEGF, etc., are processed by furin. Thus, furin expression can be used as the marker of tumor progression or as prognostic indicators [4]. Alkaloids are secondary metabolites with the wide range of pharmacological properties. Coralyne [(C₂₂H₂₂O₄N⁺) 5, 6, 7, 8, 13, 13a hexadehydro-8-methyl-2, 3, 10, 11-tetramethoxy berberinium] is a crescent-shaped, planar heterocyclic isoquinoline alkaloid with broad biological activities. It is reported to have anti-leukemic activity and relatively low toxicity [5]. Cancer bioinformatics is a field which provides classification, clustering algorithmic and soft computing techniques to understand and predict possible early markers of cancers. Bio-computation is an important approach in drug designing as it speed up the process of drug designing and provide the platform to identify novel lead compounds. Docking of the lead molecule with the receptor at the possible drug action complementarity sites are responsible for pharmaceutical effect [6]. In the present study, the binding ability of coralyne with various metastatic proteases was analyzed using in silico docking. Further, a protein-protein network was analyzed using STRING version 10 based on KEGG pathways and clustered into groups using MCL and k-mean algorithms to unfold Furin interaction proteins in cancer metastasis.

2.2 Materials and Method

2.2.1 Retrieval of Receptors and Designing of Ligand

The crystal structure of the receptors was retrieved from Protein Data Bank (PDB). The PDB IDs of serine proteases: Furin (1P8J), uPA (4MNW), matrix metallo-proteinases: MT1-MMP (1BQQ), TIMP-1 (1V96), TIMP-2 (1BR9), MMP-2 (1CK7), MMP-9 (1L6J), cysteine proteases: Cathepsin-B (2IPP), Cathepsin-S (4P6E), aspartate proteases: Cathepsin-D (1LYB). The 2D structure of coralyne and berberine was designed using Chemi-informatic software and converted to 3D structures.

2.2.2 Molecular Docking with iGEMDOCK v2.1

The proteases were docked with the ligands under docking accuracy settings (GA parameters) with binding site radius 8° A ($X = 8.3$, $Y = 8.3$ and $Z = 8.3$) $^{\circ}$ A each; population size 200; solutions 3; generation 70. The hydrophobic and electrostatic preference were set to 1.00. The empirical scoring function of iGEMDOCK was determined at: Fitness = van der Waal energy (vdW) + hydrogen bonding energy (Hbond) + electro static energy (Elec).

2.2.3 Docking with Hex v6.3

Hex docking of proteases and ligand was performed using the method of [7]. Docking was done by bringing the ligand into the vicinity of the receptor. Based on the energy minimization and 3D shape optimization, the Hex v6.3 docking control was set as follows: Correlation Type: Shape only, FFT Mode: 3D, Grid Dimension: 0.6, Solutions: 2000, Step Size: 7.5, Receptor Range: 180, Ligand Range: 180, Step Size: 7.5, Step Size: 5.5, Twist Range: 360, Scan Step: 0.8, Distance Range: 40, Sub Steps: 0.

2.2.4 Patchdock

Patchdock server (www.bioinfo3d.cs.tau.ac.il/PatchDock) was used to compute the scores of the docked complexes. The server depends on the principle of surface patch, molecular shape matching, filtering and scoring. 3D structures of proteases and ligand were submitted in PDB format with a protein-ligand parameter in Patchdock as the given input for the molecular docking. The output obtained is in the order of highly shape complementarity criteria which is given in the form of the score. The Patchdock results were evaluated for active site interactions by UCSF-chimera software [6].

2.2.5 STRING

STRING (*Search Tool for the Retrieval of Interacting Genes/Proteins*) identifies protein-protein interaction partners. This tool offers pre-computed interaction data derived from varied sources, such as high thought-put, experimental and literature data and computational predictions. The prediction methods selected for the neighborhood, gene fusion, co-occurrence, co-expression, experiments, database and text mining. Allows grouping of interacting molecules into clusters using MCL

(Markov clustering) and k-mean algorithms in the advanced mode. Therefore, this tool was used to query, retrieve and analyze the furin protein interaction network with the interactions restricted to those available for *Homosapiens*.

2.3 Results

In the present study, iGEMDOCK v2.1, hex v6.3 and patchdock were used to dock coralyne a lead target and berberine as standard with serine, cysteine, aspartic acid proteases and matrix metalloproteinases using molecular docking analysis.

2.3.1 Effect of Coralyne on Serine Proteases

Furin is a serine protease expressed by all tissues and cells. It is a membrane bound, calcium-dependent endoprotease. It plays a crucial role in the physiological function of embryogenesis, homeostasis and even in diseases such as cancer. Thus, finding potential inhibitors of furin may provide a promising approach in the treatment of cancer metastasis [8]. Urokinase-type plasminogen activator (uPA), which is involved in the conversion of plasminogen to plasmin, which directly or indirectly dissociates the extracellular matrix (ECM) via activation of (pro-MMPs), thus involved in cancer metastasis [9].

In the present study, docking of serine proteases like furin and uPA with coralyne was carried out by iGEMDOCK v2.1, hex v6.3 and patchdock (Figs. 2.1, 2.2, 2.3). The results obtained for total binding energy using iGEMDOCK was -96.95 and -91.80 kcal/mol for furin and uPA, respectively. Active site amino acids associated were: Thr 226 (-2.6), Pro 170 (-6.1), Gly 208 (-5.6), Asn 209 (-12), Asn 209 (-5.3), Ser 210 (-7.6), Gly 211 (-8); Gln 154 (-3.5), Phe 21 (-4.3), Thr 22 (-6.3), Thr 23 (-5.4), Arg 72 (-10.2) Thr 77 (-4), Gln 154 (-13.1). Berberine which was used as standard protoberberines alkaloid exhibited binding energy of -86.27 with active site amino acids as Arg 207 (-3.3), Pro 170 (-4.7), Ile 205 (-4.5), Ile 205 (-8.6) Arg 207 (-15.9), Ser 210 (-4) with furin and total binding energy of -81.39 with active site amino acids as Gly 69 (-3.5), Arg 70 (-5.1), Arg 70 (-4), Arg 70 (-4), Ser 71 (-4.2), Arg 72 (-4.7), Leu 73 (-7.4), Glu 153 (6.1), Gln 154 (-5.6), Leu 155 (-10.1) with uPA. Coralyne exhibited high affinity for furin and uPA, which may be due to presence of Thr and Gln at active site. Docking of aforesaid serine proteases using hex v6.3 reveals the binding energy as -290.06 and -281.26 for coralyne and berberine -272.19 and -234.54 kcal/mol (Table 2.1). Docking with patchdock showed score of 5784 and

Table 2.1 Docking score (kcal/mol) of coralyne and berberine with proteases

Proteases	iGEMDOCK v2.1										Hex 6.3	
	Total binding energy		Vander waal force		Hydrogen bond		E-value					Berberine
	Coralyne	Berberine	Coralyne	Berberine	Coralyne	Berberine	Coralyne	Berberine	Coralyne	Berberine	Coralyne	
Serine protease	Furin (1P8J)	-96.95	-86.27	-87.26	-13.26	-	-290.06	-	-290.06	-	-272.19	Berberine
	uPA (4MNV)	-91.80	-81.39	-67.75	-10.62	-13.64	-281.26	-13.64	-281.26	-	-234.54	Berberine
Matrix metallo proteinase	MT1-MMP (1BQQ)	-64.59	-72.89	-67.82	-4.42	-5.07	-261.17	-5.07	-261.17	-	-271.29	Berberine
	TIMP-1 (1V96)	-87.5	-92.23	-87.23	-2.94	-5.00	-251.63	-5.00	-251.63	-	-262.13	Berberine
	TIMP-2 (1BR9)	-74.52	-74.98	-63.94	-12.91	-11.04	-259.09	-11.04	-259.09	-	-263.89	Berberine
	MMP-2 (1CK7)	-83.68	-94.55	-76.79	-6.83	-1.38	-257.21	-1.38	-257.21	-	-292.92	Berberine
	MMP-9 (1L6J)	-86.76	-93.30	-90.70	-4.51	-2.60	-262.59	-2.60	-262.59	-	-297.67	Berberine
Cysteine proteases	Cathepsin-B (2IPP)	-76.47	-71.96	-65.86	-	-6.10	-269.15	-6.10	-269.15	-	-253.28	Berberine
	Cathepsin-S (4P6E)	-67.61	-65.77	-64.11	-3.50	-7.00	-218.62	-7.00	-218.62	-	-221.09	Berberine
Aspartate proteases	Cathepsin-D (1LYB)	-69.83	-69.53	-65.21	-4.61	-2.50	-208.32	-2.50	-208.32	-	-227.63	Berberine

4796 for furin and uPA with coralyne; 5222 and 5177 with berberine. These results indicate that among above mentioned serine proteases furin has higher affinity for coralyne in comparison with berberine compared to uPA protease.

2.3.2 Effect of Coralyne on Matrix Metalloproteinases

Metalloproteinases (MMPs) are involved in cancer invasion and metastasis by degrading basement membrane which is mainly carried out by Membrane-bound MMPs (MT1) degrade ECM macromolecules, such as collagen I and III, laminin, vitronectin, fibronectin and proteoglycans [10, 11]. The major endogenous regulators of MMP activities is tissue inhibitors of metalloproteinases (TIMPs). Studies on the expression of MMP and TIMP have shown that cancer progression is well associated with the expression and/or overexpression of certain MMPs and TIMP-1 [12]. Figure 2.1 demonstrate the binding energy of matrix metalloproteinases MT1-MMP (1BQQ), TIMP-1 (1V96), TIMP-2 (1BR9), MMP-2 (1CK7), MMP-9 (1L6J) with coralyne using iGEMDOCK was revealed as -64.59 (Ser 1004 (-3.3) ,

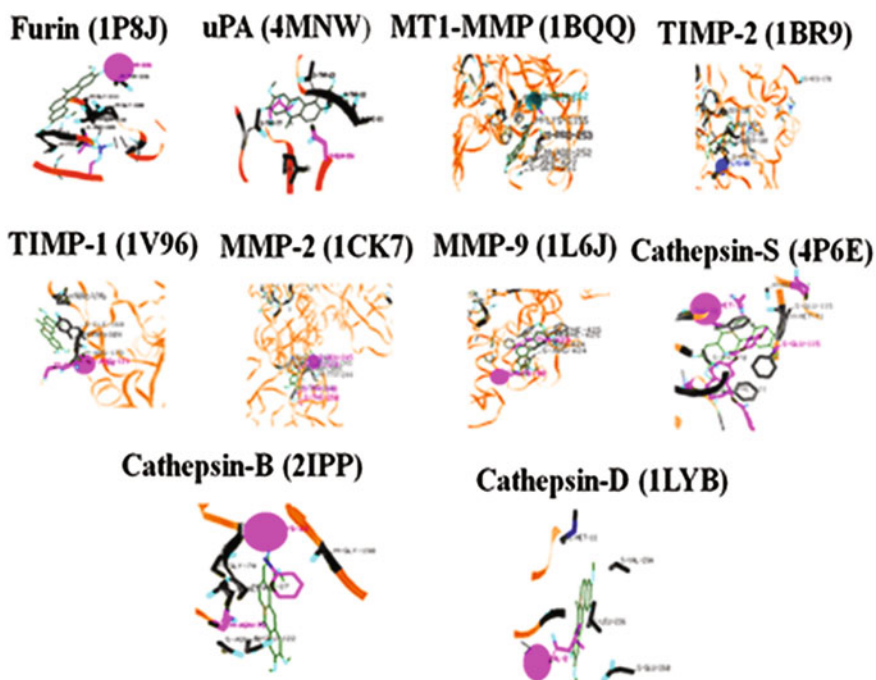


Fig. 2.1 Docking poses of proteases with coralyne using iGEMDOCK v2.1

Trp 1151 (-6.2), Asn 229 (-5.2), Tyr 261 (-6.7), Pro 1005 (-7.6), Pro 1005 (-8.2), Arg 1132 (10.9)); -87.5 (His 178 (-2.9), Lys 88 (-8.1), Ile 96 (-5.6), Ser 100 (-6.8), Ser 100 (-5.9)), -74.52 (Gln 10 (-3.4), Asn 14 (-6.9), Ser 141 (-2.5), Gln 10 (-8.5), Asn 14 (-4.5), Lys 129 (-12.1), Ile 130 (-5.5), Thr 131 (-5.4), Glu 145 (-9.8)), -83.68 (Lys 187 (-2.6), Asp 370 (-4.2), Gly 371 (-8.1), Met 373 (-7.2), Phe 389 (-6.9), Asp 392 (-11.4) and -86.76 (Tyr 52 (-2.5), Asn 38 (-7), Leu 44 (-7.6), Tyr 48 (-6.8), Arg 51 (-4.1), Tyr 52 (-5), Arg 95 (-12.1), Gly 186 (-11.4)) which was higher than berberine -72.89 (Ser 189 (-3.5), Asp 1034 (-3.1), Tyr 203 (10.6), Lys 1041 (-12.5), Phe 1067 (-5.7)), -92.23 (Cys 70 (-2.5), Ser 179 (-2.5), Thr 97 (-9.9), Cys 99 (-6.4), Ser 100 (-5.4), Asp 174 (-6.3), His 178 (-9.9)), -74.98 (Ser 7 (-3.5), Ala 15 (-2.5), Arg 102 (-2.5), Cys 3 (-4.9), Ser 4 (-5.2), Cys 100 (-5.1), -94.55 (Pro 417 (-8.8), Ala 422 (-6.6), Ile 424 (-6.5), Thr 426 (-4.7), Leu 508 (-5.9)) and -93.30 (Arg 51 (-2.7), Tyr 48 (-4.2), Tyr 52 (-4.1), Met 94 (-8.5), Pro 97 (-4.3), Gly 186 (-6)) Table 2.1. Further, results obtained from docking using hex v6.3 reveals binding energies with coralyne (-261.17, -251.63, -259.09, -257.21 and -262.59); berberine (-271.29, -262.13, -263.89 and -292.92), respectively with aforesaid matrix metalloproteinases (Table 2.1) and Fig. 2.2. Patch dock showed score of 4446, 5148, 4330, 4422 and 5162 with coralyne and 3446, 5253, 5138, 3658 and 4556 with berberine as shown in Table 2.2 and Fig. 2.3. Coralyne exhibited high binding affinity with MMPs than berberine.

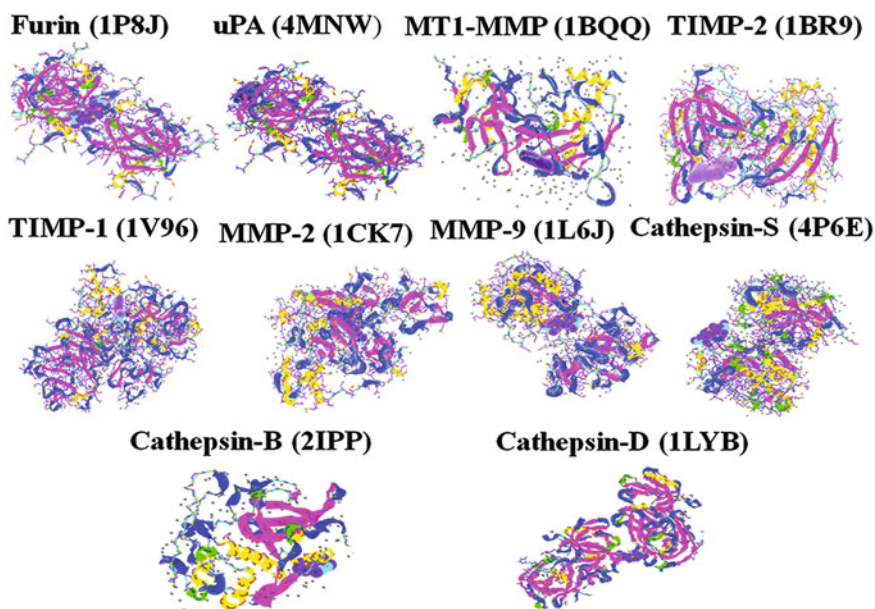


Fig. 2.2 Docking poses of proteases with coralyne using Hex v6.3

Table 2.2 Docking score of coralyne and berberine with proteases using patchdock

Proteases		Patchdock					
		Score		Area		Atomic contact energy (ACE)	
		Coralyne	Berberine	Coralyne	Berberine	Coralyne	Berberine
Serine protease	Furin (1P8J)	5278	−82.01	679.60	−76.82	−178.23	−5.07
	uPA (4MNW)	4422	−65.77	565.80	−289.48	−289.48	−7.00
Matrix metallo proteinase	MT1-MMP (1BQQ)	4446	−81.39	544.60	−67.75	−256.46	−13.64
	TIMP-1 (1V96)	4848	595.30	595.30	−87.23	−273.69	−5.00
	TIMP-2 (1BR9)	4330	−74.98	563.80	−63.94	−455.57	−11.04
	MMP-2 (1CK7)	5222	−94.55	687.70	−93.16	332.28	−1.38
Cysteine proteases	MMP-9 (1L6J)	4662	−93.30	655.60	−90.70	−411.35	−2.60
	Cathepsin-B (2IPP)	4318	−71.96	637.00	−65.86	−352.77	−6.10
	Cathepsin-S (4P6E)	4446	−86.27	544.60	−87.26	−242.61	−
Aspartate proteases	Cathepsin-D (1LYB)	5112	−69.53	670.00	−67.03	242.61	−2.50

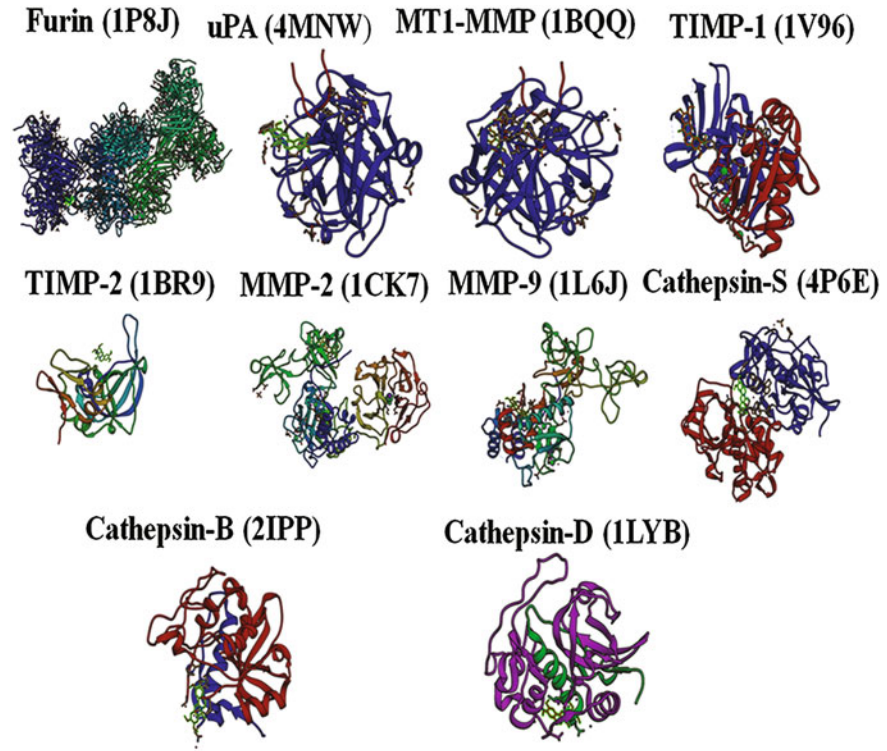


Fig. 2.3 Docking poses of proteases with coralyne using patchdock

2.3.3 *Effect of Coralyne on Cysteine and Aspartate Proteases*

Cysteine proteases are a proteolytic enzymes which are characterized by the presence of cysteine residue at an active site. They are confined to lysosome and cytosol. Reports suggested that there is a correlation between the activity of cysteine proteases and cancer metastasis as these proteases are involved in dissociation of extracellular matrix proteins [13]. Cathepsin B involved in degradation and reformation of basement membrane which may lead to cancer growth, invasion and metastasis [3]. Aspartic proteases are enzymes with two lobes bridged by a cleft containing with two aspartate residues at the catalytic site. Cathepsin-D is one of the aspartic endoprotease which is ubiquitous in distribution and confined to lysosomes. Studies suggests that variation in the expression levels of this protease may be responsible in cancer metastasis [14]. In the present study, cathepsin-B (2IPP) and cathepsin-S (4P6E) were considered for cysteine proteases and cathepsin-D (1LYB) as aspartate proteases. Figure 2.1 demonstrate the binding efficiency of coralyne with cathepsin B and S as -76.47 (Trp 30 (-5.1), Asn 72 (-4.9), Gly 73 (-9.8), His 199 (-9.8), Ala 200 (-6)), -67.61 (Lys 236 (-3.5), Ser 80 (-9.8), Ser 80(-5.3), Gln 258 (-7.6), Glu 260 (-8.7), Val 3 (-4.7), Met 307 (-7.7)) and with berberine as -71.96 (Trp 26 (-3.5), Gly 165 (-3.5), Gly 165 (-3.5), Ser (213 (-2.5)), -65.77 (Ser 7 (-3.5), Ala 15 (-2.5), Phe 70 (-4.6), Met 71 (-7.4)) with iGEMDOCK and -290.06 and -253.28 with coralyne; -253.28 and -221.09 with berberine (Table 2.1) using hex v6.3 Fig. 2.2 and score of 4318 and 4446 for coralyne; 3970 and 5440 with berberine using patchdock (Table 2.2) and Fig. 2.3. Aspartic acid proteases exhibited -69.83 (Leu 67 (-2.5), Ser 89 (-4.7), Gln 98 (-3.5), Glu 260 (-6.4), Met 72 (-3.5), Val 294 (-7.6), Leu 236 (-2.5)) for coralyne; -69.53 (Cys 25 (-3.8), Trp 26 (-3.5), Gly 165 (-3.5), Ser (213 (-2.5), Gly 69 (-7.1), Phe 70 (-4.6), Met 71 (-7.4), Val 258 (-5.3), Leu 236 (-2.5)), for berberine using iGEMDOCK as shown in Fig. 2.1. Docking with hex Fig. 2.2 reveals coralyne exhibited binding affinity as -208.32 and -227.63 for berberine (Table 2.1) and score of 5112 and 4700 for coralyne and berberine (Table 2.2) using patchdock Fig. 2.3. More the negative value higher is the affinity, thus among all the proteases serine proteases exhibited high binding energy and as the binding affinity of coralyne was higher than berberine it can be used as antimetastatic alkaloid targeting proteases.

2.3.4 *Identification of Furin Interaction Protein Partners*

As furin is having high binding affinity with coralyne, its interaction partners were identified using STRING. The results showed that furin exhibited interaction with Notch, TIMP, MMPs and VEGF which are involved in regulation development of cell. Further, protein-protein interacting clusters were analyzed using K-mean [15].

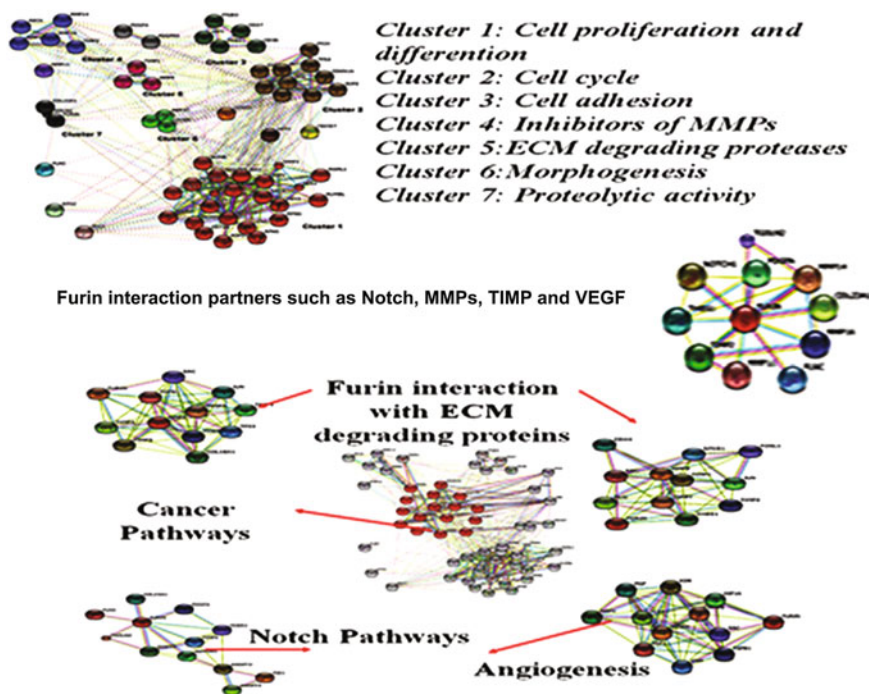


Fig. 2.4 Furin clustering using K-mean and interaction with metastatic proteins using KEGG pathway

The results showed that Cluster 1 is involved in cell proliferation and differentiation, cluster 2 regulates cell cycle, cluster 3 is involved in cell adhesion, cluster 4 are the inhibitors of MMPs, cluster 5 are ECM degrading proteases, cluster 6 is involved in morphogenesis and cluster 7 are involved proteolysis (Fig. 2.4).

2.4 Discussion

In the present study, it was analyzed that coralyne (ligand) exhibited high negative binding energy with furin, a serine protease, this indicates the high binding affinity between coralyne and furin. Reports suggest that furin which is an important member of the family of pro-protein processing enzymes and is highly expressed in various tumors. Thus, protein-protein network was analyzed to unfold its interaction proteins in cancer metastasis using STRING, which offers pre-computed interaction data derived from varied sources, such as, high thought-put, experimental data, literature data and computational predictions. It was analyzed that furin exhibited direct interaction with proteins involved in tumor development, invasion and metastasis including Notch, TIMP, MMPs, VEGF. Thus, furin can be an

important target for controlling cancer metastasis. Clustering of various interacting proteins with furin was performed by k-mean algorithms. The clusters were characterized as proteins involved in cell development, differentiation, cell cycle, invasion and metastasis. Further, using KEGG pathway proteins involved in cancer pathway where furin showed direct or indirect interactions were predicted.

2.5 Conclusion

A significant interaction of coralyne with furin, a serine protease was observed compared to other proteases. Further, Notch, TIMP, MMPs and VEGF which are involved in cancer progression was predicted as protein interaction partners of furin. Thus, this study may be useful for evaluating the effect of coralyne on various mechanisms of different cancers, both in vitro and in vivo models and also to identify furin as the drug target.

Acknowledgements The present research work was supported by UGC, (New Delhi) with file number (No.F.15-1/2013-2014/PDFWM-2013-2014-GE-AND-12376 (SA-II)) for Post-Doctoral Fellow for Woman. I would like to thanks, GITAM University for providing lab facilities.

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Computational Intelligence Techniques in Health Care

Lakshmi, P.V.; Zhou, W.; Satheesh, P. (Eds.)

2016, VII, 100 p. 23 illus., 21 illus. in color., Softcover

ISBN: 978-981-10-0307-3