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Identification and mechanism of peptides with activity promoting osteoblast proliferation from bovine lactoferrin



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ABSTRACT

Lactoferrin (LF) is a basic glycoprotein and a dominant functional component from the whey protein in bovine milk. As a novel bone growth factor, it can fulfill its biological functions of preventing osteoporosis by regulating the growth and metabolic processes of bone. However, it has not been clarified that LF plays a role of osteo-genesis in a form of molecular fragments after enzymatic digestion. In this study, a novel peptide with osteoblast proliferation activity, ENLPEKADRDQYEL, was identified using UPLC-Q-TOF-MS/MS and Mascot analysis. The mechanism of promoting proliferation of osteoblast activity was also analyzed by molecular docking. Results demonstrated that ENLPEKADRDQYEL can significantly promote the proliferation of osteoblasts. The main interaction forces of ENLPEKADRDQYEL with epidermal growth factor receptor (EGFR) were the hydrophobic and hydrogen bonding. ENLPEKADRDQYEL had similar target domain (Lys13-Leu14-Thr15-Gln16-Leu98-Ser99-Ser418) with the key structure of EGFR compared with epidermal growth factor (EGF). This work established a theoretical foundation for the peptide from lactoferrin used as a functional component in functional dairy products.

1. Introduction

Osteoporosis is an age-related skeletal disorder leading to low bone mass and weakening bone microarchitecture (Lerner, 2006). It occurs due to unbalanced bone remodeling and altered functions of the osteoclasts and osteoblasts (Chim et al., 2013; McClung et al., 2013). To treat and prevent bone loss, there are several drugs available either suspend or prevent bone loss or bone reconstruction. Latest osteoporosis treatments are anti-resorptive medicines which inhibit osteoclastic bone resorption but not promote new bone formation, including risedronate, alendronic acid, bisphosphonate, zoledronic acid and alendronate, have been widely used in the clinical treatment to reduce the risk of fracture (Amso et al., 2016; Calabria et al., 2016; Eriksen, Diez-Perez, & Boonen, 2014; Park et al., 2014). However, these treatments may cause several side effects, including a risk of an inflamed esophagus, nausea and abdominal pain. Therefore, the discovery of novel bone-anabolic agents with less toxicity, greater safety and the ability to increase bone strength, bone mass and potentially reversing structural damage are attracting more attentions.

In recent years, a variety of osteogenic peptides with less side effects to establish an alternative strategy were reported as natural alternative bioactive peptides. A peptide named osteogenic growth peptide (OGP)

was reported to serve as an important bone growth factor (Pigossi, Medeiros, Saska, Cirelli, & Scarel-Caminaga, 2016). OGP is a 14-amino acid motif (ALKRQGRTLYGFGG) with a primary structure identical from C-terminus of histone H4. It has been verified that OGP had promoting effect on bone regeneration, mainly in stimulating the differentiation, proliferation, and matrix mineralization of osteoblast via MAPK signal pathway (Maia et al., 2014). Atsuhiro et al. also reported a synthetic peptide (NSVNSKIPKACCVPTELSAI), relating to residues 68-87 of BMP-2, recruited osteocalcin-positive osteoblasts to induce ectopic calcification (Saito, Suzuki, Ogata, Ohtsuki, & Tanihara, 2005). Besides, the principal cytokines such as insulinlike growth factor-1 (IGF-1), transforming growth factors $-\alpha$ and $-\beta$ (TGF- α and TGF- β), epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) (Bikle & Wang, 2011; Cinque et al., 2015; Crane & Cao, 2014; Plonka et al., 2017; Tian, Guo, Zhuang, Chu, & Zhang, 2014) showed a critical role in all aspects of skeletal development and bone remodeling (Soon et al., 2015). Although being the most potential therapy currently available, the high cost of isolation and labor-intensive of purification has caused cytokines hard to be a potent treatment.

Milk proteins are regarded to be the most important source of bioactive peptides, which attract more growing interests in the field of

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Table 1
Peptides profile of lactoferrin hydrolysate identified by UPLC-Q-TOF-MS/MS

1 747.4008 746.3963 50.01 7 APVDAFK 256-262 lactoferrin 2 805.3898 804.3865 35.43 7 ETAERVK 352.358 lactoferrin 3 439.7389 877.4658 56.41 8 DSALGFLR 352.350 lactoferrin 4 516.7676 1031.5247 64.65 9 ETAEEVKAR 352.360 lactoferrin 5 355.1799 1062.5206 47.18 8 NINREDR 333.342 lactoferrin 6 540.786 1079.5611 25.19 10 VDSALYLGSR 333.342 lactoferrin 8 374.5603 1120.6604 30.11 9 YLTILKNLR 343.351 lactoferrin 10 61.63148 1231.6167 25.61 11 APKGLTWNSLK 461-471 lactoferrin 11 63.2929 1264.5764 59.09 11 EPYKOTSGAFK 206-216 lactoferrin 13 439.2214 1316.7452 53.53 12<	No.	<i>m/z</i> meas.	Mr. calc.	Scores	Length	Amino Acid Sequence	Range	Protein
2 805.3898 804.3865 35.43 7 ETAERVK 352.368 lactoferrin 4 516.7676 1031.5247 64.65 9 ETAEEVKAR 321.322 lactoferrin 5 355.1799 1062.5206 47.18 8 NINREDFR 582.589 lactoferrin 6 540.786 1079.5611 25.19 10 VISALYLGSR 333.342 lactoferrin 7 549.2539 1096.4978 57.48 9 YUTTIKNIK 349.351 lactoferrin 8 374.5603 1120.6604 30.11 9 YUTTIKNIK 349.351 lactoferrin 10 616.8148 1231.6197 45.61 11 ANEGLWNSLK 206-216 lactoferrin 12 645.3371 1288.6663 7.95 11 SUGESPEGOR 30-312 lactoferrin 14 660.8357 1319.6622 56.01 12 SUGESPEGOR 30-312 lactoferrin 15 677.8224 1350.778 166.77 <t< td=""><td>1</td><td>747.4008</td><td>746.3963</td><td>50.01</td><td>7</td><td>APVDAFK</td><td>256-262</td><td>lactoferrin</td></t<>	1	747.4008	746.3963	50.01	7	APVDAFK	256-262	lactoferrin
3 439,7899 87,4658 56,41 8 DSALGFUR 321,328 lactoferrin 4 516,7676 103,52206 47,18 8 NLNREDFR 352,360 lactoferrin 6 540,766 1079,5611 25,19 10 VDALUGSR 333,342 lactoferrin 7 549,2539 1066,4797 57,48 9 YYGYTGAFR 542,550 lactoferrin 8 374,5603 1120,6604 30.11 9 YUTKIKNIR 349,351 lactoferrin 9 594,8117 118,6146 27,59 10 NLETAEEVK 461,471 lactoferrin 11 633,292 1264,5764 59,09 11 EYYGYGAFR 278-288 lactoferrin 13 439,9214 1316,7452 53,53 12 LRPVAREIVGTK 278-288 lactoferrin 14 666,8357 1319,6622 56,01 12 SPUGSPFQDR 304,315 lactoferrin 15 677,8224 133,6533 68,59 11 EXYGYTGAFR 540-550 lactoferrin 16 454,2451 <td>2</td> <td>805.3898</td> <td>804.3865</td> <td>35.43</td> <td>7</td> <td>ETAEEVK</td> <td>352-358</td> <td>lactoferrin</td>	2	805.3898	804.3865	35.43	7	ETAEEVK	352-358	lactoferrin
4 516,7676 1031,5247 64.65 9 FLEVKAR 352.360 lactoferrin 5 355.1799 1062,2206 47.18 8 NLNREDFR 582.559 lactoferrin 6 540.786 1079,5611 25.19 10 VDSALYLGSR 333.342 lactoferrin 7 549.2539 1006,4978 57.48 9 YUTTKANR 343.351 lactoferrin 9 594.8117 1187.6146 27.59 10 NIRCHTARLEVK 343.351 lactoferrin 11 613.8148 1231.017 45.61 11 ANEGLTWNSLK 461.471 lactoferrin 12 645.371 1288.6663 77.95 11 SPGKEDLWK 206-216 lactoferrin 13 439.9214 1316.622 55.01 12 RPVAEUGTK 93.104 lactoferrin 14 660.8357 1319.6622 56.01 12 RPVAEUGTKR 940.40 lactoferrin 15 677.8224 1353.03 66.99 11 EKYGYTGAR 204.406.471 lactoferrin 16 454.24	3	439.7389	877.4658	56.41	8	DSALGFLR	321-328	lactoferrin
5 355.1799 1062.5206 47.18 8 NLREPFR 582-589 lactoferrin 6 540.786 1079.5611 25.19 10 VDSALYLGSR 333.342 lactoferrin 7 549.2539 1096.4978 57.48 9 YGYTGAR 542.550 lactoferrin 8 374.5603 1120.6604 30.11 9 YLTKNLR 343.351 lactoferrin 10 616.8148 1231.6197 45.61 11 ANCUTWNSLK 349.351 lactoferrin 11 633.2929 1264.5764 59.09 11 EVPGYSGAFK 206-216 lactoferrin 13 439.9214 1316.7452 53.53 12 RNCUTWNSLK 278-288 lactoferrin 14 660.8357 1316.662 56.01 12 SFQLGSPFCQR 304.15 lactoferrin 15 677.8224 1353.6353 68.59 11 EKYGYGTGAR 460.471 lactoferrin 16 454.2411 1359.746 32.91 12 KNCGUTWALEYAEW 349.360 lactoferrin 16 45	4	516.7676	1031.5247	64.65	9	ETAEEVKAR	352-360	lactoferrin
6 540,786 1079,5611 25.19 10 VDSALVLGSR 333-342 lactoferrin 7 549,2539 1096,4978 57.48 9 YUGYTGAFR 542.550 lactoferrin 9 574,8603 1120,6604 30.11 9 YLTTLKNLR 343.351 lactoferrin 9 594,8117 1187,6146 27.59 10 NLRETAEEVK 349-358 lactoferrin 11 616,8148 123,6197 45.61 11 NARECLTWNSLK 206-216 lactoferrin 12 645,3371 1288,6663 77.95 11 SVDCKRDLJWKK 278-288 lactoferrin 13 439,9214 1316,6752 53.53 12 LRPVAREVTKK 39-104 lactoferrin 14 660,8357 1319,6622 56.01 12 SFQLFGSPFQQR 304.315 lactoferrin 15 677,8224 1333,6353 68.59 11 EXPYGYTGAFR 540-550 lactoferrin 16 454.2441 1359,716 32.91 12 KANEGLTWNSLK 40-471 lactoferrin 17	5	355.1799	1062.5206	47.18	8	NLNREDFR	582-589	lactoferrin
7 549.2539 1096.4978 57.48 9 YYGYTGAFR 542.550 lactoferrin 8 374.5603 1120.6604 30.11 9 YLTLKNLR 343.351 lactoferrin 9 594.8117 1187.6146 27.59 10 NLRETAEFVK 349.358 lactoferrin 10 616.8148 1231.6197 45.61 11 EPYEGYGAFK 206-216 lactoferrin 12 645.3371 1288.6663 77.95 11 SVDGKEDLWK 278-288 lactoferrin 13 439.9214 1316.7452 53.03 12 ERVYGYTGAFR 93-104 lactoferrin 14 660.3837 1310.6622 56.01 12 SFQLFGSPEQG 304.315 lactoferrin 15 677.8224 1335.633 68.59 11 EXYGYTGAFR 540-550 lactoferrin 16 454.2441 1359.7146 32.91 12 KANEGLWNSLK 640-471 lactoferrin 17 681.8386 1361.6688 84.43 12 ONLETAEEVKAR 494.93.01 lactoferrin 18	6	540.786	1079.5611	25.19	10	VDSALYLGSR	333-342	lactoferrin
8 374, 5603 1120, 6604 30.11 9 VLTLKNLR 343, 351 lactoferrin 9 594, 8117 1187, 6146 27, 59 10 NLRETAEEVK 349, 358 lactoferrin 11 616, 8148 1231, 6197 45, 61 11 ANEGLTWNSLK 206, 216 lactoferrin 12 645, 3371 1288, 6663 77, 95 11 SVDCKEDLWK 278, 228 lactoferrin 13 439, 9214 1316, 7452 53, 53 12 LRPVAAEIYCTK 93, 104 lactoferrin 14 606, 3357 1319, 6622 56, 01 12 SPQLFGSPPQQR 304, 315 lactoferrin 15 677, 8224 1353, 333 68, 59 11 EKYGYGTGAFR 606, 404, 120, 131 lactoferrin 16 454, 2441 1359, 7146 32, 91 12 VLRETAEEVKAR 49, 360 lactoferrin 17 681, 8386 1361, 668 84, 43 12 OL CVTGAFR 28, 39, 30 lactoferrin 164 <td>7</td> <td>549.2539</td> <td>1096.4978</td> <td>57.48</td> <td>9</td> <td>YYGYTGAFR</td> <td>542-550</td> <td>lactoferrin</td>	7	549.2539	1096.4978	57.48	9	YYGYTGAFR	542-550	lactoferrin
9 594,8117 1187,6146 27,59 10 NLRTAEVK 349-358 lactoferrin 10 616,8148 1231,6197 45,61 11 ANEGLTWNSLK 461-471 lactoferrin 12 645,3371 1288,6663 77,95 11 SVDGKEDLWK 278-288 lactoferrin 13 439,9214 1316,7452 55,01 12 SPQLFGSPPGQR 304-315 lactoferrin 14 660,8357 1319,6622 56,01 12 SPQLFGSPPGQR 304-315 lactoferrin 15 677,8224 1353,6533 68.9 11 EKYYGYTGAFR 400-550 lactoferrin 16 454,2441 1359,7146 32.91 12 GNNQLDQLQGR 120-131 lactoferrin 17 681,8386 1360,688 84.43 12 GNNQLDQLQGR 120-131 lactoferrin 18 412,2887 1447,716 99.15 13 ANEGLTWNSLKDK 64-473 lactoferrin 19 472,5897 141,47528	8	374.5603	1120.6604	30.11	9	YLTTLKNLR	343-351	lactoferrin
10 616.8148 1231.6197 45.61 11 ANEGLTWNSLK 461.471 lactoferrin 11 633.2929 1264.5764 59.09 11 EYPGYSGAFK 206.216 lactoferrin 13 439.9214 1316.7452 53.53 12 LRPVAAEIYGTK 39.104 lactoferrin 14 660.8357 1319.6622 56.01 12 SPQLFGSPFQQR 304.315 lactoferrin 15 677.8224 1353.6333 68.59 11 EKYGYGYGARR 50.050 lactoferrin 16 454.2441 1359.7146 32.91 12 KANEGLTWNSLK 460.471 lactoferrin 17 681.8386 1361.6688 84.43 12 GSNFQLDQLQGR 120.131 lactoferrin 18 461.2685 1380.7878 106.77 12 QVLHQALFGK 628.639 lactoferrin 21 745.8855 1489.7637 112.14 13 KGSNFQLDQLQGR 119.131 lactoferrin 22 496.409 1718.8034 <td>9</td> <td>594.8117</td> <td>1187.6146</td> <td>27.59</td> <td>10</td> <td>NLRETAEEVK</td> <td>349-358</td> <td>lactoferrin</td>	9	594.8117	1187.6146	27.59	10	NLRETAEEVK	349-358	lactoferrin
11 632.929 1264.5764 59.09 11 EPYEGYSGAFK 206-216 lactoferrin 12 645.3371 1288.6663 77.95 11 SVDGKEDLIWK 278-288 lactoferrin 13 439.9214 1316.7452 53.53 12 LRPVAAEIYCTK 30-3104 lactoferrin 14 660.8357 1319.6622 56.01 12 SPQLFGSPPGQR 304-315 lactoferrin 15 677.8224 1353.6353 68.59 11 EXYGYTGAFR 540-550 lactoferrin 16 454.241 1359.7146 32.91 12 GNNFQLDQLQGR 120-131 lactoferrin 17 681.8386 1361.6688 84.43 12 QVLLHQQALFGK 628-639 lactoferrin 18 461.2685 1380.7878 106.77 12 QVLLHQQALFGK 628-639 lactoferrin 20 492.5861 1474.7416 99.15 13 ANEGLTWNSLKDK 461-473 lactoferrin 21 745.8855 1489.7637 112.14 13 KGSMPGLDQLQGR 101-5328 lactoferrin <	10	616.8148	1231.6197	45.61	11	ANEGLTWNSLK	461-471	lactoferrin
12 645.3371 1288.6663 77.95 11 SVDGKEDLIWK 278-288 lactoferrin 13 439.9214 1316.7452 53.53 12 LRPVAAEIYGTK 93-104 lactoferrin 14 660.8357 1319.6622 50.01 12 SPQLFGSPPGQR 304.315 lactoferrin 15 677.8224 1353.6353 68.59 11 EKYYGYTGAFR 540-550 lactoferrin 16 454.2441 1359.7146 32.91 12 KANEGLTWNSLK 460-471 lactoferrin 17 681.8386 1361.6688 84.43 12 GNFQLDQLQGR 120-131 lactoferrin 18 461.2685 1380.7878 106.77 12 QVLHQQALFGK 628-639 lactoferrin 20 492.5861 1474.7416 99.15 13 ANEGLTWNSLKDK 461-473 lactoferrin 21 745.8855 1489.7637 112.14 13 KGSNPCLDQLQGR 19-131 lactoferrin 22 498.9468 1493.8242 112.83 13 DLLFKDSALGFLR 316.328 lactoferrin	11	633.2929	1264.5764	59.09	11	EPYFGYSGAFK	206-216	lactoferrin
13 439.9214 1316.7452 53.53 12 LRPVAAEIYGTK 93.104 lactoferrin 14 660.8357 1319.6622 56.01 12 SPQLIGSPPCQR 304.315 lactoferrin 15 677.8224 1353.6353 68.9 11 EKYYGYTGAFR 540.550 lactoferrin 16 454.2441 1359.7146 32.91 12 KANEGLTWNSLK 460.471 lactoferrin 17 681.8386 1361.6688 84.43 12 GNFQLDQLQGR 120.131 lactoferrin 18 461.2685 1380.7878 106.77 12 QVLHQQALFGK 628-639 lactoferrin 19 472.5897 1414.7528 49.94 12 NLRETAEEVKAR 349.360 lactoferrin 21 745.8855 1489.7637 112.14 13 AKGSNFQLDQLQGR 119.131 lactoferrin 22 498.9468 1493.8242 112.83 13 DLIFKDSALGFLR 316.325 lactoferrin 23 80.409 171.8034 46.29 14 ENPEKADRDQVEL 235-248 lactoferrin <	12	645.3371	1288.6663	77.95	11	SVDGKEDLIWK	278-288	lactoferrin
14 660.8357 1319.6622 56.01 12 SFQLFGSPPGQR 304-315 lactoferrin 15 677.8224 1353.6353 68.59 11 EKYYGYTGAFR 540-550 lactoferrin 16 454.2441 1359.714 32.91 12 KANEGLTWNSLK 404.1 lactoferrin 17 681.8386 1361.6688 84.43 12 GSNFQLDQLQGR 120-131 lactoferrin 18 461.2685 1380.7878 106.77 12 QVLlHQQALFGK 628-639 lactoferrin 19 472.5897 1414.7528 49.94 12 NLETAEUKAR 494-360 lactoferrin 20 492.5861 1447.716 9.915 13 ANEGITWNSLKDK 414.73 lactoferrin 21 745.8855 1489.7637 112.14 13 MCSNFQLDQLQGR 119-131 lactoferrin 23 860.409 1718.8034 46.29 14 ENLPEKADRDQYEL 235-248 lactoferrin 24 502.614 1504.824 62.87 14 PSKUDSAL/LGSR 329-342 lactoferrin	13	439.9214	1316.7452	53.53	12	LRPVAAEIYGTK	93-104	lactoferrin
15 677.8224 1353.6353 68.59 11 EKYYGYTGAFR 540-550 lactoferrin 16 454.2441 1350.7146 32.91 12 KANEGLTWNSLK 460-471 lactoferrin 17 681.8386 1361.6688 84.43 12 GSNFQLDQLQGR 120-131 lactoferrin 18 461.2685 1380.787 106.77 12 QVLLHQALFGK 628.639 lactoferrin 19 472.5897 1414.7528 49.94 12 NLRETAEEVKAR 349.360 lactoferrin 20 492.5861 1474.7416 99.15 13 ANEGLTWNSLKDK 461-473 lactoferrin 21 745.8855 1489.7637 112.14 13 KGSNFQLDQLQGR 119-131 lactoferrin 23 860.409 1718.8034 46.29 14 ENLPEKADRDQYEL 235-248 lactoferrin 24 502.614 1548.249 62.87 14 IPSKVDSALYLGSR 302-315 lactoferrin 25 51.9375 1562.7954 42.47 14 ESPQLHYAVAVK 105-118 lactoferrin	14	660.8357	1319.6622	56.01	12	SFQLFGSPPGQR	304-315	lactoferrin
16 454.2441 1359.7146 32.91 12 KANEGLTWNSLK 460-471 lactoferrin 17 681.8386 1361.6688 84.43 12 GSNFQLDQLQCR 120.131 lactoferrin 18 461.2685 1380.7878 106.77 12 QVLLHQQALFGK 628-639 lactoferrin 19 472.5897 1414.7528 49.94 12 NLETAELVKAR 349-360 lactoferrin 20 492.5861 1474.7416 99.15 13 ANEGLTWNSLKDK 461-473 lactoferrin 21 745.8855 1489.7637 112.14 13 CSSNFQLDQLQCR 119.131 lactoferrin 22 496.0409 1718.8034 46.29 14 ENLPEADRDQYEL 235-248 lactoferrin 24 502.614 1504.8249 62.87 14 IPSKVDSALYGSR 302.315 lactoferrin 25 511.9375 1562.7954 42.47 14 SRSPQLFGSPPCQR 302.315 lactoferrin 26 531.2738 1602.8365 58.38 14 ANEGLTWNSLKDKK 461.474 lactoferrin </td <td>15</td> <td>677.8224</td> <td>1353.6353</td> <td>68.59</td> <td>11</td> <td>EKYYGYTGAFR</td> <td>540-550</td> <td>lactoferrin</td>	15	677.8224	1353.6353	68.59	11	EKYYGYTGAFR	540-550	lactoferrin
17 681.8386 1361.6688 84.43 12 GSNFQLDQLQGR 120-131 lactoferrin 18 461.2685 1380.7878 106.77 12 QVLLHQQALFGK 628-639 lactoferrin 19 472.5897 1414.7528 49.94 12 NLRETAEEVKAR 349-360 lactoferrin 20 492.5861 1474.7416 99.15 13 ANEGLTWNSLKDK 461.473 lactoferrin 21 745.8855 1489.7637 112.14 13 NEGLTWNSLKDK 461.473 lactoferrin 22 498.9468 1493.8242 112.83 13 DLLFKDSALGFLR 316-328 lactoferrin 23 860.409 1718.8034 46.29 14 ENLPEKADRDQYEL 235-248 lactoferrin 24 502.614 1504.8249 62.87 14 IPSKVDSALYEGSR 329-342 lactoferrin 25 521.9375 1562.7954 42.47 14 SRSFQLFGSPFQRQR 302-315 lactoferrin 26 531.2738 1590.8042 75.79 14 ESPQTHYYAVAVK 105-118 lactoferrin	16	454.2441	1359.7146	32.91	12	KANEGLTWNSLK	460-471	lactoferrin
18 461.2685 1380.7878 106.77 12 QVLLHQQALFGK 628-639 lactoferrin 19 472.5897 1414.7528 49.94 12 NLRETAEEVKAR 349.360 lactoferrin 20 492.5861 1474.7416 99.15 13 ANEGLTWNSLKDK 461.473 lactoferrin 21 745.8855 1493.6247 112.14 13 KGSNFQLDQLQGR 119-131 lactoferrin 22 498.9468 1493.8242 112.83 13 DLLFKDSALGFLR 235-248 lactoferrin 23 860.409 1718.8034 46.29 14 ENLPEKADRDQYEL 235-248 lactoferrin 24 502.614 1504.8249 62.87 14 ISSPQLFGSPPGQR 302-315 lactoferrin 26 531.2738 1500.8042 75.79 14 ESPQTHYYAVAVVK 105-118 lactoferrin 28 540.2914 1617.8587 54.95 14 KGSNFQLDQLQGRK 119-132 lactoferrin 29 914.4367 1826.866 18.51 18 GEADALNLDGGYIYTAGK 406-423 lactoferrin <td>17</td> <td>681.8386</td> <td>1361.6688</td> <td>84.43</td> <td>12</td> <td>GSNFQLDQLQGR</td> <td>120-131</td> <td>lactoferrin</td>	17	681.8386	1361.6688	84.43	12	GSNFQLDQLQGR	120-131	lactoferrin
19 472.5897 1414.7528 49.94 12 NLRETAEEVKAR 349-360 lactoferrin 20 492.5861 1474.7416 99.15 13 ANEGLTWNSLKDK 461.473 lactoferrin 21 745.8855 1489.7637 112.14 13 KGSNFQLDQLQGR 119.131 lactoferrin 22 498.9468 1493.8242 112.83 13 DLLFKDSALGFLR 316-328 lactoferrin 23 860.409 1718.8034 46.29 14 ENLPEKADRDQYEL 235-248 lactoferrin 24 502.614 1504.8249 62.87 14 IPSKVDSALYLGSR 329-342 lactoferrin 25 521.9375 1562.7954 42.47 14 SRSFQLFGSPPGQR 302-315 lactoferrin 26 531.2738 1590.8042 75.79 14 ESPQTHYYAVAVK 105-118 lactoferrin 27 535.2843 1602.8365 58.38 14 ANEGLTWNSLKDKK 461-474 lactoferrin 29 914.4367 1826.8686 18.51 18 GEADALNLDGGYIYTAGK 406-423 lactoferrin </td <td>18</td> <td>461.2685</td> <td>1380.7878</td> <td>106.77</td> <td>12</td> <td>QVLLHQQALFGK</td> <td>628-639</td> <td>lactoferrin</td>	18	461.2685	1380.7878	106.77	12	QVLLHQQALFGK	628-639	lactoferrin
20 492.5861 1474.7416 99.15 13 ANEGLTWNSLKDK 461-473 lactoferrin 21 745.8855 1489.7637 112.14 13 KGSNFQLDQLQGR 119-131 lactoferrin 22 498.9468 1493.8242 112.83 13 DLLFKDSALGFLR 316-328 lactoferrin 23 860.409 1718.8034 46.29 14 ENLPEKADRDQYEL 235-248 lactoferrin 24 502.614 1504.8249 62.87 14 IPSKVDSALYLGSR 329-342 lactoferrin 25 521.9375 1562.7954 42.47 14 SRSPQIFGSPPGQR 302-315 lactoferrin 26 531.2738 1500.8042 75.79 14 ESPQTHYYAVAVK 105-118 lactoferrin 27 535.2843 1602.8365 58.38 14 ANEGLTWNSLKDKK 461-474 lactoferrin 28 540.2914 1617.8587 54.95 14 KGSNFQLDQLQGRK 119-132 lactoferrin 30 646.3452 1936.0207 17.61 17 SFQLFGSPPGQRDLLFK 304-320 lactoferrin<	19	472.5897	1414.7528	49.94	12	NLRETAEEVKAR	349-360	lactoferrin
21 745.8855 1489.7637 112.14 13 KGSNFQLDQLQGR 119-131 lactoferrin 22 498.9468 1493.8242 112.83 13 DLLFKDSALGFLR 316-328 lactoferrin 23 860.409 1718.8034 46.29 14 ENLPEKADRDQYEL 235-248 lactoferrin 24 502.614 1504.8249 62.87 14 IPSKVDSALYLGSR 329-342 lactoferrin 25 521.9375 1562.7954 42.47 14 SRSPQLFGSPPGQR 302-315 lactoferrin 26 531.2738 1500.8042 75.79 14 ESPQTHYYAVAVVK 105-118 lactoferrin 28 540.2914 1617.8587 54.95 14 ANEGLTWNSLKDKK 461-474 lactoferrin 29 914.4367 1826.8686 18.51 18 GEADALNLDGGYIYTAGK 406-423 lactoferrin 30 646.3452 1936.0207 17.61 17 SFQLFGSPPGQRDLLFK 304-320 lactoferrin 31 747.3697 236.42801 38.65 22 DSALGFLIPSKVDSALYLGSR 321-342 <td< td=""><td>20</td><td>492.5861</td><td>1474.7416</td><td>99.15</td><td>13</td><td>ANEGLTWNSLKDK</td><td>461-473</td><td>lactoferrin</td></td<>	20	492.5861	1474.7416	99.15	13	ANEGLTWNSLKDK	461-473	lactoferrin
22 498.9468 1493.8242 112.83 13 DLLFKDSALGFLR 316-328 lactoferrin 23 860.409 1718.8034 46.29 14 ENLPEKADRDQYEL 235-248 lactoferrin 24 502.614 1504.8249 62.87 14 IPSKVDSALYLGSR 329-342 lactoferrin 25 521.9375 1562.7954 42.47 14 SRSFQLFGSPPGQR 302-315 lactoferrin 26 531.2738 1590.8042 75.79 14 ESPQTHYYAVVK 105-118 lactoferrin 27 535.2843 1602.8365 58.38 14 ANEGLTWNSLKDKK 461-474 lactoferrin 28 540.2914 1617.8587 54.95 14 KGSNFQLDQLQGRK 119-132 lactoferrin 30 646.3452 1936.0207 17.61 17 SFQLFGSPPGQRDLLFK 304-320 lactoferrin 31 747.3697 236.4201 38.65 22 DSALGFLIPSKVDSALYLGSR 321-342 lactoferrin 32 592.0757<	21	745.8855	1489.7637	112.14	13	KGSNFQLDQLQGR	119-131	lactoferrin
23 860.409 1718.8034 46.29 14 ENLPEKADRDQYEL 235-248 lactoferrin 24 502.614 1504.8249 62.87 14 IPSKVDSALYLGSR 329.342 lactoferrin 25 521.9375 1562.7954 42.47 14 SRSFQLFGSPPGQR 302.315 lactoferrin 26 531.2738 1590.8042 75.79 14 ESPQTHYYAVAVVK 105-118 lactoferrin 27 535.2843 1602.8365 58.38 14 ANEGLTWNSLKDKK 461-474 lactoferrin 28 540.2914 1617.8587 54.95 14 KGSNFQLDQLQGRK 119-132 lactoferrin 30 646.3452 1936.0207 17.61 17 SFQLFGSPPGQRDLLFK 304-320 lactoferrin 31 747.3697 2230.9943 71.36 21 KADAVTLDGGMVFEAGRDPYK 407-423 lactoferrin 32 592.0757 2364.2801 38.65 22 DSALGFLIPSKVDSALYLGSR 321-342 lactoferrin 33 <td< td=""><td>22</td><td>498.9468</td><td>1493.8242</td><td>112.83</td><td>13</td><td>DLLFKDSALGFLR</td><td>316-328</td><td>lactoferrin</td></td<>	22	498.9468	1493.8242	112.83	13	DLLFKDSALGFLR	316-328	lactoferrin
24 502.614 1504.8249 62.87 14 IPSKVDSALYLGSR 329-342 lactoferrin 25 521.9375 1562.7954 42.47 14 SRSFQLFGSPPGQR 302-315 lactoferrin 26 531.2738 1590.8042 75.79 14 ESPQTHYYAVAVVK 105-118 lactoferrin 27 535.2843 1602.8365 58.38 14 ANEGLTWNSLKDKK 461-474 lactoferrin 28 540.2914 1617.8587 54.95 14 KGSNFQLDQCGRK 119-132 lactoferrin 29 914.4367 1826.8686 18.51 18 GEADALNLDGGYIYTAGK 406-423 lactoferrin 30 646.3452 1936.0207 17.61 17 SFQLFGSPPGQRDLLFK 304-320 lactoferrin 31 747.3697 239.0943 71.36 21 KADAVTLDGGMVFEAGRDPYK 407-423 lactoferrin 32 592.0757 2364.2801 38.65 22 DSALGFLIPSKVDSALYLGSR 321-342 lactoferrin 33	23	860.409	1718.8034	46.29	14	ENLPEKADRDQYEL	235-248	lactoferrin
25 521.9375 1562.7954 42.47 14 SRSFQLFGSPPGQR 302-315 lactoferrin 26 531.2738 1590.8042 75.79 14 ESPQTHYYAVAVVK 105-118 lactoferrin 27 535.2843 1602.8365 58.38 14 ANEGLTWNSLKDKK 461-474 lactoferrin 28 540.2914 1617.8587 54.95 14 KGSNFQLDQLQGRK 119-132 lactoferrin 29 914.4367 1826.8666 18.51 18 GEADALNLDGGYIYTAGK 406-423 lactoferrin 30 646.3452 1936.0207 17.61 17 SFQLFGSPPGQRDLLFK 304-320 lactoferrin 31 747.3697 2239.0943 71.36 21 KADAVTLDGGMVFAGRDPYK 407-423 lactoferrin 32 592.0757 2364.2801 38.65 22 DSALGFLRPISKVDSALYLGSR 312.342 lactoferrin 33 672.3552 2685.4013 116.68 24 LGGRPTYEEYLGTEYVTAIANLKK 670-693 lactoferrin 34	24	502.614	1504.8249	62.87	14	IPSKVDSALYLGSR	329-342	lactoferrin
26 531.2738 1590.8042 75.79 14 ESPQTHYYAVAVVK 105-118 lactoferrin 27 535.2843 1602.8365 58.38 14 ANEGLTWNSLKDKK 461-474 lactoferrin 28 540.2914 1617.8587 54.95 14 KGSNFQLDQLQGRK 119-132 lactoferrin 29 914.4367 1826.8686 18.51 18 GEADALNLDGGYIYTAGK 406-423 lactoferrin 30 646.3452 1936.0207 17.61 17 SFQLFGSPPGQRDLLFK 304-320 lactoferrin 31 747.3697 2239.0943 71.36 21 KADAVTLDGGMVFAGRDPYK 407-423 lactoferrin 32 592.0757 2364.2801 38.65 22 DSALGFLIPSKVDSALYLGSR 321-342 lactoferrin 33 672.3552 2685.4013 116.68 24 LGGRPTYEFVLGTEYVTAIANLKK 670-693 lactoferrin 34 699.8738 2795.4759 24.41 25 SFQLFGSPPGQRDLLFKDSALGFLR 304-328 lactoferrin	25	521.9375	1562.7954	42.47	14	SRSFQLFGSPPGQR	302-315	lactoferrin
27 535.2843 1602.8365 58.38 14 ANEGLTWNSLKDKK 461-474 lactoferrin 28 540.2914 1617.8587 54.95 14 KGSNFQLDQLQGRK 119-132 lactoferrin 29 914.4367 1826.8686 18.51 18 GEADALNLDGGYIYTAGK 406-423 lactoferrin 30 646.3452 1936.0207 17.61 17 SFQLFGSPPGQRDLLFK 304-320 lactoferrin 31 747.3697 2239.0943 71.36 21 KADAVTLDGGMVFEAGRDPYK 407-423 lactoferrin 32 592.0757 2364.2801 38.65 22 DSALGFLIPSKVDSALYLGSR 321-342 lactoferrin 33 672.3552 2685.4013 116.68 24 LGGRPTYEFLGTEYVTAIANLKK 670-693 lactoferrin 34 699.8738 2795.4759 24.41 25 SFQLFGSPPGQRDLLFKDSALGFLR 304-328 lactoferrin 35 1095.2505 3282.7475 104.6 30 SAGWIIPMGILRPYLSWTESLEPLQGAVAK 141-170 lactoferrin </td <td>26</td> <td>531.2738</td> <td>1590.8042</td> <td>75.79</td> <td>14</td> <td>ESPQTHYYAVAVVK</td> <td>105-118</td> <td>lactoferrin</td>	26	531.2738	1590.8042	75.79	14	ESPQTHYYAVAVVK	105-118	lactoferrin
28 540.2914 1617.8587 54.95 14 KGSNFQLDQLQGRK 119-132 lactoferrin 29 914.4367 1826.8686 18.51 18 GEADALNLDGGYIYTAGK 406-423 lactoferrin 30 646.3452 1936.0207 17.61 17 SFQLFGSPPGQRDLLFK 304-320 lactoferrin 31 747.3697 2239.0933 71.36 21 KADAVTLDGGMVFEAGRDPYK 407-423 lactoferrin 32 592.0757 2364.2801 38.65 22 DSALGFLIPSKVDSALYLGSR 321-342 lactoferrin 33 672.3552 2685.4013 116.68 24 LGGRPTYEFLGTEYVTALANLKK 670-693 lactoferrin 34 699.8738 2795.4759 24.41 25 SFQLFGSPPGQRDLLFKDSALGFLR 304-328 lactoferrin 35 1095.2505 3282.7475 104.6 30 SAGWIIPMGILRPYLSWTESLEPLQGAVAK 141-170 lactoferrin	27	535.2843	1602.8365	58.38	14	ANEGLTWNSLKDKK	461-474	lactoferrin
29 914.4367 1826.8686 18.51 18 GEADALNLDGGYIYTAGK 406-423 lactoferrin 30 646.3452 1936.0207 17.61 17 SFQLFGSPPGQRDLLFK 304.320 lactoferrin 31 747.3697 2239.0943 71.36 21 KADAVTLDGGWVFEAGRDPYK 407-423 lactoferrin 32 592.0757 2364.2801 38.65 22 DSALGFLRIPSKVDSALYLGSR 321-342 lactoferrin 33 672.3552 2685.4013 116.68 24 LGGRPTYEEYLGTEYVTAIANLKK 670-693 lactoferrin 34 699.8738 2795.4759 24.41 25 SFQLFGSPPGQRDLLFKDSALGFLR 304-328 lactoferrin 35 1095.2505 3282.7475 104.6 30 SAGWIIPMGILRPYLSWTESLEPLQGAVAK 141-170 lactoferrin	28	540.2914	1617.8587	54.95	14	KGSNFQLDQLQGRK	119-132	lactoferrin
30 646.3452 1936.0207 17.61 17 SFQLFGSPPGQRDLLFK 304-320 lactoferrin 31 747.3697 2239.0943 71.36 21 KADAVTLDGGMVFEAGRDPYK 407-423 lactoferrin 32 592.0757 2364.2801 38.65 22 DSALGFLRIPSKVDSALYLGSR 321-342 lactoferrin 33 672.3552 2685.4013 116.68 24 LGGRPTYEEYLGTEYVTAIANLKK 670-693 lactoferrin 34 699.8738 2795.4759 24.41 25 SFQLFGSPPGQRDLLFKDSALGFLR 304-328 lactoferrin 35 1095.2505 3282.7475 104.6 30 SAGWIIPMGILRPYLSWTESLEPLQGAVAK 141-170 lactoferrin	29	914.4367	1826.8686	18.51	18	GEADALNLDGGYIYTAGK	406-423	lactoferrin
31 747.3697 2239.0943 71.36 21 KADAVTLDGGMVFEAGRDPYK 407-423 lactoferrin 32 592.0757 2364.2801 38.65 22 DSALGFLRIPSKVDSALYLGSR 321-342 lactoferrin 33 672.3552 2685.4013 116.68 24 LGGRPTYEEYLGTEYVTAIANLKK 670-693 lactoferrin 34 699.8738 2795.4759 24.41 25 SFQLFGSPPGQRDLLFKDSALGFLR 304-328 lactoferrin 35 1095.2505 3282.7475 104.6 30 SAGWIIPMGILRPYLSWTESLEPLQGAVAK 141-170 lactoferrin	30	646.3452	1936.0207	17.61	17	SFQLFGSPPGQRDLLFK	304-320	lactoferrin
32 592.0757 2364.2801 38.65 22 DSALGFLRIPSKVDSALYLGSR 321-342 lactoferrin 33 672.3552 2685.4013 116.68 24 LGGRPTYEEYLGTEYVTAIANLKK 670-693 lactoferrin 34 699.8738 2795.4759 24.41 25 SFQLFGSPPGQRDLLFKDSALGFLR 304-328 lactoferrin 35 1095.2505 3282.7475 104.6 30 SAGWIIPMGILRPYLSWTESLEPLQGAVAK 141-170 lactoferrin	31	747.3697	2239.0943	71.36	21	KADAVTLDGGMVFEAGRDPYK	407-423	lactoferrin
33 672.3552 2685.4013 116.68 24 LGGRPTYEEYLGTEYVTAIANLKK 670-693 lactoferrin 34 699.8738 2795.4759 24.41 25 SFQLFGSPPGQRDLLFKDSALGFLR 304-328 lactoferrin 35 1095.2505 3282.7475 104.6 30 SAGWIIPMGILRPYLSWTESLEPLQGAVAK 141-170 lactoferrin	32	592.0757	2364.2801	38.65	22	DSALGFLRIPSKVDSALYLGSR	321-342	lactoferrin
34 699.8738 2795.4759 24.41 25 SFQLFGSPPGQRDLLFKDSALGFLR 304-328 lactoferrin 35 1095.2505 3282.7475 104.6 30 SAGWIIPMGILRPYLSWTESLEPLQGAVAK 141-170 lactoferrin	33	672.3552	2685.4013	116.68	24	LGGRPTYEEYLGTEYVTAIANLKK	670-693	lactoferrin
35 1095.2505 3282.7475 104.6 30 SAGWIIPMGILRPYLSWTESLEPLQGAVAK 141-170 lactoferrin	34	699.8738	2795.4759	24.41	25	SFQLFGSPPGQRDLLFKDSALGFLR	304-328	lactoferrin
	35	1095.2505	3282.7475	104.6	30	SAGWIIPMGILRPYLSWTESLEPLQGAVAK	141-170	lactoferrin



Fig. 1. Docking for the interaction of epidermal growth factor (EGF) and epidermal growth factor receptor (EGFR) (PDB:11VO) used for validation. (a) 3D structure of EGF and EGFR complex as a surface image, generated by the surface menu of Discovery Studio 2017 software based on the PDB database. (b) Active site of EGFR and the selective site used for molecule docking. (c) Structure of EGF. (d) EGF and EGFR compound after docking.

health-beneficial functional foods (Mohanty, Mohapatra, Misra, & Sahu, 2016). Lactoferrin (LF), a dominant functional component from the whey protein in bovine milk, is a concerned glycoprotein for its regulatory effect on bone cells that results cured in some pathological conditions, such as osteoporosis (Gao et al., 2016; Li, Zhu, & Hu, 2015). In the previous study, lactoferrin was reported that it had impact on osteogenesis showing protective effects on bone resorption of ovariectomized rats, and had a significantly promoting effect on JNK1/2, ERK1/2 and p38 mRNA expression (Du et al., 2011). Therefore, lactoferrin as an excellent origin to produce bioactive peptides for osteoporosis has aroused considerable attention.

Molecular docking works based on the "lock-key principle" which represented the function of ligand and receptor, simulating interaction between the receptor and small molecular biological ligand (Rawendra, Chang, Chen, Huang, & Hsu, 2013). Molecular docking predicts the ligand best model which suitable to the binding site of a macromolecular target. It serves a quick and high-throughput screening method to produce bioactive peptide from protein according the protein-ligand bindings principle (Thomsen & Christensen, 2006).

In the present study, the molecular docking method was used to screen and reveal the mechanism of osteogenic peptides from lactoferrin hydrolysates. Trypsin was utilized to hydrolyze lactoferrin to obtain a peptide mixture and the sequence of peptides in hydrolysates were identified with UPLC-Q-TOF-MS/MS and Mascot analysis. The interaction of these peptides and epidermal growth factor receptor (EGFR) was analyzed by Molecular docking software, and the peptides with better affinity to EGFR were screened. The potential peptides with osteoblast proliferation promotion activity were synthesized based on Download English Version:

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