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Insights Into Cytokine–Receptor Interactions from Cytokine Engineering
Spangler et al.

Table 1. Examples of cytokine engineering reported in the literature

Cytokine	Mutation(s)	Method	Design Objective	Functional Effect	Reference
IL-1	Chimera of IL-1Ra and IL-1β	Yeast display	High affinity for IL-1R1	Inhibition of dry-eye disease	(1)
IL-2	Q126K; Q126L; Q126D (Mouse analogs)	Site-directed mutagenesis	Lowered affinity for γc	Reduced complex internalization; IL-2 partial agonist	(2)
IL-2	L18M, L19S	Cassette mutagenesis	Lowered affinity for IL-2Rβ and γc	Increased recycling of IL-2; Enhanced T cell activation	(3)
IL-2	T51P	Site-directed mutagenesis	Lowered affinity for IL-2Rβ	Reduced complex internalization; Higher activity	(4)
IL-2	N88R	Site-directed mutagenesis	Lowered affinity for IL-2Rβ	Specific T cell activation	(5)
IL-2	N29S, Y31H, K35R, K48E, V69A, N71R, Q74P, N88D, I89V (Mutant 2-4)	Yeast display	High affinity for IL-2Rα	Increased recycling of IL-2; Enhanced T cell activation	(6)
IL-2	P65A, V69A, Q74P; K35L, M39V; P65A; V69A	Phage display	High affinity for IL-2Rα	Not tested	(7)
IL-2	V69A, Q74P, I128T, V91R, Q126T	Yeast display	High affinity for IL-2Rα, low affinity for IL-2Rβ	T_{reg} inhibition	(8)
IL-2	L80F, R81D, L85V, I86V, I92F (Mutant H9)	Yeast display	High affinity for IL-2Rβ	Anti-tumor activity	(9)
IL-2	R38A, F42A, Y45A, E62A	Site-Directed Mutagenesis	IL-2Rα binding inhibition	Melanoma	(10)
IL-2	D20T	Site-directed mutagenesis	Lowered affinity for IL-2Rβ; Fused to anti-DNA tumor-targeting antibody	Anti-tumor activity	(11)
IL-3	A101, V116	Site-directed mutagenesis	High affinity for IL-3Rα	Enhanced biological potency	(12)
IL-3	>20 mutations; See reference for details	Site-directed mutagenesis	High affinity for IL-3Rα	Enhanced hematopoietic activity	(13)
IL-4	Y124D	Site-directed mutagenesis	Lowered affinity for for γc/IL13Rα	IL-4 antagonist	(14)
IL-4	R121E	Site-directed mutagenesis	γc binding specificity	Enhanced Th cell differentiation	(15)
IL-4	*De novo* 4 helix bundle	-	Binds IL-4Rα	IL-4 antagonist	(16)
IL-4	K117R, T118V, R121Q, E122S, Y124W, S125F, S128G, S129A	Yeast display	γc/IL-13Rα1 binding specificity	Enhanced Th and dendritic cell differentiation	(17)
IL-5	E12K	Site-directed mutagenesis	Lowered affinity for IL-5Rα	IL-5 antagonist	(18)
IL-6	S176R	Site-directed mutagenesis	High affinity for IL-6Rα	IL-6 agonist (transcriptional enhancement)	(19)
IL-6	Y31D, G35F, S118F, V121D, Q175I, S176R, Q183A, S176R	Site-directed mutagenesis	Lowered affinity for gp130, high affinity for IL-6Rα	IL-6 antagonist	(20)
IL-6	Q175I, S176R, Q183A	Site-directed mutagenesis	High affinity IL-6Rα	IL-6 agonist (transcriptional enhancement)	(20)
IL-6	Q159E, T162P, F170L, S176A and substitution of human IL-6 residues 43-55 with mouse IL-6 residues	Site-directed mutagenesis	Lowered affinity for gp130, high affinity for IL-6Rα	IL-6 antagonist	(21)
IL-6	Y31D, G35F, S118R, V121D, L57D, E59F, N60W, Q75Y, S76K, Q175I, S176R, Q183A	Site-directed mutagenesis	Lowered affinity for gp130, high affinity for IL-6Rα	IL-6 antagonist	(22)
IL-6	A56D, I57D, E59F, N60W, Q75Y, S76K	Phage display	High affinity IL-6Rα	IL-6 agonist (proliferation enhancement)	(23)
IL-6	Fusion of IL-6 and IL-6Rα	Single-chain fusion	High affinity for gp130	IL-6 agonist (hematopoietic cell expansion enhancement)	(24)
LIF	Q25A, S28A, Q32A	Site-directed mutagenesis	Lowered affinity for gp130	LIF antagonist	(26)
LIF	P53G,N54L,N55L,L56Q,D57G, Q29A,G124R	Phage display	High affinity for LIFR, Loweredaffinity for gp130	LIF antagonist	(27)
CNTF	F152A,K155A,S166D,Q167H	Site-directed mutagenesis	Lowered affinity for CNTFRα	CNTF antagonist	(28)
IL-7	W143A	Site-directed mutagenesis	Lowered affinity for γc chain	IL-7 antagonist	(29)
IL-13	R112D	Site-directed mutagenesis	High affinity for IL-13Rα	IL-13 agonist	(30)
IL-13	R112D, E12K	Site-directed mutagenesis	High affinity for IL-13Rα1, lowered affinity for IL-4Rα	IL-13 antagonist	(31)
IL-13	E12K, R66D, S69D	Site-directed mutagenesis	Lowered affinity for IL-4Rα	Inhibition of Glioblastoma multiforme	(32)
IL-13	K105R	Site-directed mutagenesis	High affinity for IL-13Rα2	Inhibition of Glioblastoma multiforme	(33)
IL-15	Q101D, Q108D	Site-directed mutagenesis	High affinity for IL-15Rα	IL-15 antagonist (proliferation and infiltration obstruction)	(34)
IL-15	Fusion of IL-15 and truncated IL-15Rα (Residues 1-66)	Single-chain fusion	High affinity for IL-2Rβ/γc heterodimer	IL-15 agonist (proliferation enhancement and prevention of apoptosis)	(35)
IL-15	N72D	Site-directed mutagenesis	High affinity for IL-2Rβ	IL-15 agonist (proliferation enhancement and prevention of apoptosis)	(36)
IFN	IFNα2 with the IFNα8 C-terminal region	Site-directed mutagenesis	High affinity for IFNAR2	Antiviral, antiproliferative activity enhancement	(37)
IFN	>20 mutations; See reference for details	Gene shuffling	High affinity for IFNAR1	High antiviral activity, low antiproliferative activity	(38)
IFN	H57Y, E58N, Q61S	Phage display	High affinity for IFNAR1	Antiviral, antiproliferative activity enhancement	(39)
IFN	R120E	Site-directed mutagenesis	Lowered affinity for IFNAR1	IFN antagonist	(40)
IFN	IFNα2 with R120E and IFNα8CTail	Site-directed mutagenesis	Lowered affinity for IFNAR1, High affinity for IFNAR2	IFN super antagonist	(40, 41)
GH	R167N,D171S,E174S,F176Y,I179T	Phage display	High affinity for receptor	Not tested	(42)
Prolactin	S61A, D68N, Q73L, K190R, G129R	Scintillation proximity assay	High affinity sitel/Low affinity site II for ProlactinR	Prolactin antagonist	(43)
Leptin	D23L, L39A, D40A, F41A	Yeast display	High affinity for LeptinR site I/low affinity for site II	Leptin antagonist	(44)
EPO	EPO mimetic peptides	Phage display	Dimerization of EpoR	EPO agonist	(45)
EPO	R130A	Site-directed mutagenesis	Lowered affinity for EpoR	EPO antagonist	(46)
EPO	EPO agonistic antibodies	Lentiviral libraries	Dimerization of EpoR	EPO agonist	(47)
TPO	TPO mimetic peptides	Phage display	Dimerization of TpoR	TPO agonist	(48)
TPO	TpoR agonistic diabodies	Retroviral library	Dimerization of TpoR	TPO agonist	(49)
TPO	TpoR agonistic antibodies	Lentiviral library	Dimerization of TpoR	TPO agonist	(50)
GM-CSF	E21R	Site-directed mutagenesis	Lowered affinity for βc	GM-CSF antagonist	(51)
EGF	Y13G	Site-directed mutagenesis	Enhancement of EGF/EGFR recycling	EGF agonist	(52)
EGF	Not provided	Gene shuffling	High affinity for EGFR and increased kinase activity	EGF agonist	(53)
EGF	S4P, E5K, D11E, H16N, E24G, A25T, K28R, I38V, W50L, E51A	Yeast display	High affinity for EGFR	Not tested	(54)
EGF	D17G, E24K, L26V, K28R, K48T, E51G, L52P; D3Y, L8P, H10Y, M21R, K28S, I38A, K48R, E51G, L52R	Yeast display	Increased EGFR k_{on}	EGF agonist	(55)
EGF	D3G, I38A, W49R; E5K, L8P, G12E, E23V, K28R, I38T, K48R, E51K	Cell-free protein synthesis array	Increased EGF potency (proliferation/migration assays)	EGF agonist	(56)
HGF	K65E, Q95R, N127D, K132N, K137R, K170E, Q173R, N193D (Mutant M2.2)	Yeast display	High affinity for Met receptor and enhancement of stability	HGF partial agonist	(57)
GHRH	P1Y, P2R, T6F, R8A, M9R, R10Y, Y11H, Y12R, F27L, T28R, N29R, S30R	Site-directed mutagenesis	High affinity for GHRH-R	Inhibition of Glioblastoma multiforme	(58)
FGF	C16S, C83S, C117S	Site-directed mutagenesis	Enhancement of stability and mitogenic activity	FGF agonist	(59)
FGF	C47A, H93G	Site-directed mutagenesis	Enhancement of stability and mitogenic activity	FGF agonist	(60)
FGF	K12C, P134C	Site-directed mutagenesis	Enhancement of stability and mitogenic activity	FGF agonist	(61)
KGF	Deletion of residues 1-23	Site-directed mutagenesis	Enhancement of stability	Not tested	(62)
IGF	E3A, F49A	Site-directed mutagenesis	Lowered affinity for IGFBP-1, wild type affinity for IGFBP-3	Increased IGF bioavailability and plasma half-life	(63)
PDGF	S129C; R85S, K86N, R87G on mouse PDGF-A	Site-directed mutagenesis	Disruption of dimer formation	Inhibition of PDGF-A activity	(64, 65)
PDGF	E156K, Y157P, V158H, R159Q, K160G, K161Q, P162H	Site-directed mutagenesis	Lowered affinity for PDGF-R	Inhibition of 3T3 cell colony formation	(66)
PDGF	Fusion with a Collagen Binding Domain	Site-directed mutagenesis	Targeting to collagen scaffold	Demonstrate an increase in fibroblast growth rate	(67)
SCF	Fusion with TPO Mimetic Petide	Site-directed mutagenesis	Costimulation by TPO and SCF	Increased cell proliferation	(68)
SCF	F63C, V49L/F63L, A165C	Site-directed mutagenesis	Alterered monomer/dimer distribution	Altered cell proliferation	(69)
SCF	Fusion with M-CSF	Site-directed mutagenesis	Costimulation by M-CSF and SCF	Increased cell proliferation	(70)

Abbreviations:
IL=Interleukin; LIF=Leukemia Inhibitory Factor; CNTF=Ciliary Neurotrophic Factor; IFN=Interferon; GH=Growth Hormone; EPO=Erythropoeitin; TPO=Thrombopoietin; GM-CSF=Granulocyte-Macrophage Colony-Stimulating Factor, EGF=Epidermal Growth Factor; HGF=Hepatocyte Growth Factor; GHRH=Growth-Hormone-Releasing Hormone; FGF=Fibroblast Growth Factor; Keratinocyte Growth Factor; IGF=Insulin-Like Growth Factor; PDGF=Platelet-Derived Growth Factor; SCF=Stem Cell Factor; M-CSF=Macrophage Colony-Stimulating Factor

Online-Only Material Literature Cited:

1 Hou J, Townson SA, Kovalchin JT, Masci A, Kiner O, Shu Y, King BM, Schirmer E, Golden K, Thomas C, Garcia KC, Zarbis-Papastoitsis G, Furfine ES, Barnes TM. 2013. Design of a superior cytokine antagonist for topical ophthalmic use. Proc Natl Acad Sci U S A 110: 3913-8
2 Imler JL, Zurawski G. 1992. Receptor binding and internalization of mouse interleukin-2 derivatives that are partial agonists. J Biol Chem 267: 13185-90
3 Berndt WG, Chang DZ, Smith KA, Ciardelli TL. 1994. Mutagenic analysis of a receptor contact site on interleukin-2: preparation of an IL-2 analog with increased potency. Biochemistry 33: 6571-7
4 Chang DZ, Wu Z, Ciardelli TL. 1996. A point mutation in interleukin-2 that alters ligand internalization. J Biol Chem 271: 13349-55
5 Shanafelt AB, Lin Y, Shanafelt MC, Forte CP, Dubois-Stringfellow N, Carter C, Gibbons JA, Cheng SL, Delaria KA, Fleischer R, Greve JM, Gundel R, Harris K, Kelly R, Koh B, Li Y, Lantz L, Mak P, Neyer L, Plym MJ, Rozcniak S, Serban D, Thrift J, Tsuchiyama L, Wetzel M, Wong M, Zolotorev A. 2000. A T-
6 Rao BM, Driver I, Lauffenburger DA, Wittrup KD. 2005. High-affinity CD25-binding IL-2 mutants potently stimulate persistent T cell growth. Biochemistry 44: 10696-701
7 Thanos CD, DeLano WL, Wells JA. 2006. Hot-spot mimicry of a cytokine receptor by a small molecule. Proc Natl Acad Sci U S A 103: 15422-7
8 Liu DV, Maier LM, Hafler DA, Wittrup KD. 2009. Engineered interleukin-2 antagonists for the inhibition of regulatory T cells. J Immunother 32: 887-94
9 Levin AM, Bates DL, Ring AM, Krieg C, Lin JT, Su L, Moraga I, Raeber ME, Bowman GR, Novick P, Pande VS, Fathman CG, Boyman O, Garcia KC. 2012. Exploiting a natural conformational switch to engineer an interleukin-2 'superkine'. Nature 484: 529-33
10 Carmenate T, Pacios A, Enamorado M, Moreno E, Garcia-Martinez K, Fuente D, Leon K. 2013. Human IL-2 mutein with higher antitumor efficacy than wild type IL-2. J Immunol 190: 6230-8
11 Gillessen S, Gnad-Vogt US, Gallerani E, Beck J, Sessa C, Omlin A, Mattiacci MR, Liedert B, Kramer D, Laurent J, Speiser DE, Stupp R. 2013. A phase I dose-escalation study of the immunocytokine EMD 521873 (Selectikine) in patients with advanced solid tumours. Eur J Cancer 49: 35-44
12 Lopez AF, Shannon MF, Barry S, Phillips JA, Cambareri B, Dottore M, Simmons P, Vadas MA. 1992. A human interleukin 3 analog with increased biological and binding activities. Proc Natl Acad Sci U S A 89: 11842-6
13 Thomas JW, Baum CM, Hood WF, Klein B, Monahan JB, Paik K, Staten N, Abrams M, McKearn JP. 1995. Potent interleukin 3 receptor agonist with selectively enhanced hematopoietic activity relative to recombinant human interleukin 3. Proc Natl Acad Sci U S A 92: 3779-83
14 Kruse N, Tony HP, Sebald W. 1992. Conversion of human interleukin-4 into a high affinity antagonist by a single amino acid replacement. EMBO J 11: 3237-44
15 Shanafelt AB, Forte CP, Kasper JJ, Sanchez-Pescador L, Wetzel M, Gundel R, Greve JM. 1998. An immune cell-selective interleukin 4 agonist. Proc Natl Acad Sci U S A 95: 9454-8
16 Laporte SL, Forsyth CM, Cunningham BC, Miercke LJ, Akhavan D, Stroud RM. 2005. De novo design of an IL-4 antagonist and its structure at 1.9 A. Proc Natl Acad Sci U S A 102: 1889-94
17 Juntilla IS, Creusot RJ, Moraga I, Bates DL, Wong MN, Alonso MN, Suhoski MM, Lupardus P, Meier-Schellersheim M, Engelman E, Utz PJ, Fathman CG, Paul WE, Garcia KC. 2012. Redirecting cell-type specific cytokine responses through structure-based engineering of interleukin-4 superkines. Nature
18 McKinnon M, Page K, Uings IJ, Banks M, Fattah D, Proudfoot AE, Graber P, Arod C, Fish R, Wells TN, Solari R. 1997. An interleukin 5 mutant distinguishes between two functional responses in human eosinophils. J Exp Med 186: 121-9
19 Savino R, Lahm A, Giorgio M, Cabibbo A, Tramontano A, Ciliberto G. 1993. Saturation mutagenesis of the human interleukin 6 receptor-binding site: implications for its three-dimensional structure. Proc Natl Acad Sci U S A 90: 4067-71
20 Savino R, Ciapponi L, Lahm A, Demartis A, Cabibbo A, Toniatti C, Delmastro P, Altamura S, Ciliberto G. 1994. Rational design of a receptor super-antagonist of human interleukin-6. Embo J 13: 5863-70
21 Cabibbo A, Sporeno E, Toniatti C, Altamura S, Savino R, Paonessa G, Ciliberto G. 1995. Monovalent phage display of human interleukin (hIL)-6: selection of superbinder variants from a complex molecular repertoire in the hIL-6 D-helix. Gene 167: 41-7
22 Ehlers M, de Hon FD, Bos HK, Horsten U, Kurapkat G, van De Leur HS, Grotzinger J, Wollmer A, Brakenhoff JP, Rose-John S. 1995. Combining two mutations of human interleukin-6 that affect gp130 activation results in a potent interleukin-6 receptor antagonist on human myeloma cells. J Biol Chem 270:
23 Sporeno E, Savino R, Ciapponi L, Paonessa G, Cabibbo A, Lahm A, Pulkki K, Sun RX, Toniatti C, Klein B, Ciliberto G. 1996. Human interleukin-6 receptor super-antagonists with high potency and wide spectrum on multiple myeloma cells. Blood 87: 4510-9
24 Toniatti C, Cabibbo A, Sporena E, Salvati AL, Cerretani M, Serafini S, Lahm A, Cortese R, Ciliberto G. 1996. Engineering human interleukin-6 to obtain variants with strongly enhanced bioactivity. EMBO J 15: 2726-37
25 Fischer M, Goldschmitt J, Peschel C, Brakenhoff JP, Kallen KJ, Wollmer A, Grotzinger J, Rose-John S. 1997. I. A bioactive designer cytokine for human hematopoietic progenitor cell expansion. Nat Biotechnol 15: 142-5
26 Hudson KR, Vernallis AB, Heath JK. 1996. Characterization of the receptor binding sites of human leukemia inhibitory factor and creation of antagonists. J Biol Chem 271: 11971-8
27 Fairlie WD, Uboldi AD, McCoubrie JE, Wang CC, Lee EF, Yao S, De Souza DP, Mifsud S, Metcalf D, Nicola NA, Norton RS, Baca M. 2004. Affinity maturation of leukemia inhibitory factor and conversion to potent antagonists of signaling. J Biol Chem 279: 2125-34
28 Di Marco A, Gloaguen I, Graziani R, Paonessa G, Saggio I, Hudson KR, Laufer R. 1996. Identification of ciliary neurotrophic factor (CNTF) residues essential for leukemia inhibitory factor receptor binding and generation of CNTF receptor antagonists. Proc Natl Acad Sci U S A 93: 9247-52
29 Cosenza L, Rosenbach A, White JV, Murphy JR, Smith T. 2000. Comparative model building of interleukin-7 using interleukin-4 as a template: a structural hypothesis that displays atypical surface chemistry in helix D important for receptor activation. Protein Sci 9: 916-26
30 Oshima Y, Joshi BH, Puri RK. 2000. Conversion of interleukin-13 into a high affinity agonist by a single amino acid substitution. J Biol Chem 275: 14375-80
31 Oshima Y, Puri RK. 2001. A novel interleukin 13 (IL-13) antagonist that blocks the biological activity of human IL-13 in immune and nonimmune cells. FASEB J 15: 1469-71
32 Nash KT, Thompson JP, Debinski W. 2001. Molecular targeting of malignant gliomas with novel multiply-mutated interleukin 13-based cytotoxins. Crit Rev Oncol Hematol 39: 87-98
33 Madhankumar AB, Mintz A, Debinski W. 2004. Interleukin 13 mutants of enhanced avidity toward the glioma-associated receptor, IL13Ralpha2. Neoplasia 6: 15-22
34 Kim YS, Maslinski W, Zheng XX, Stevens AC, Li XC, Tesch GH, Kelley VR, Strom TB. 1998. Targeting the IL-15 receptor with an antagonist IL-15 mutant/Fc gamma2a protein blocks delayed-type hypersensitivity. J Immunol 160: 5742-8
35 Mortier E, Quemener A, Vusio P, Lorenzen I, Boublik Y, Grotzinger J, Plet A, Jacques Y. 2006. Soluble interleukin-15 receptor alpha (IL-15R alpha)-sushi as a selective and potent agonist of IL-15 action through IL-15R beta/gamma. Hyperagonist IL-15 x IL-15R alpha fusion proteins. J Biol Chem 281: 1612-9
36 Zhu X, Marcus WD, Xu W, Lee HI, Han K, Egan JO, Yovandich JL, Rhode PR, Wong HC. 2009. Novel human interleukin-15 agonists. J Immunol 183: 3598-607
37 Slutzki M, Jaitin DA, Yehezkel TB, Schreiber G. 2006. Variations in the unstructured C-terminal tail of interferons contribute to differential receptor binding and biological activity. J Mol Biol 360: 1019-30
38 Brideau-Andersen AD, Huang X, Sun SC, Chen TT, Stark D, Sas IJ, Zadik L, Dawes GN, Guptill DR, McCord R, Govindarajan S, Roy A, Yang S, Gao J, Chen YH, Skartved NJ, Pedersen AK, Lin D, Locher CP, Rebbapragada I, Jensen AD, Bass SH, Nissen TL, Viswanathan S, Foster GR, Symons JA, Patten
39 Kalie E, Jaitin DA, Abramovich R, Schreiber G. 2007. An interferon alpha2 mutant optimized by phage display for IFNAR1 binding confers specifically enhanced antitumor activities. J Biol Chem 282: 11602-11
40 Pan M, Kalie E, Scaglione BJ, Raveche ES, Schreiber G, Langer JA. 2008. Mutation of the IFNAR-1 receptor binding site of human IFN-alpha2 generates type I IFN competitive antagonists. Biochemistry 47: 12018-27
41 Levin D, Schneider WM, Hoffmann HH, Yarden G, Busetto AG, Manor O, Sharma N, Rice CM, Schreiber G. 2014. Multifaceted activities of type I interferon are revealed by a receptor antagonist. Sci Signal 7: ra50
42 Lowman HB, Bass SH, Simpson N, Wells JA. 1991. Selecting high-affinity binding proteins by monovalent phage display. Biochemistry 30: 10832-8
43 Liu Y, Gong W, Breinholt J, Norskov-Lauritsen L, Zhang J, Ma Q, Chen J, Panina S, Guo W, Li T, Zhang J, Kong M, Liu Z, Mao J, Christensen L, Hu S, Wang L. 2011. Discovery of the improved antagonistic prolactin variants by library screening. Protein Eng Des Sel 24: 855-60
44 Shpilman M, Niv-Spector L, Katz M, Varol C, Solomon G, Ayalon-Soffer M, Boder E, Halpern Z, Elinav E, Gertler A. 2011. Development and characterization of high affinity leptins and leptin antagonists. J Biol Chem 286: 4429-42
45 Wrighton NC, Farrell FX, Chang R, Kashyap AK, Barbone FP, Mulcahy LS, Johnson DL, Barrett RW, Jolliffe LK, Dower WJ. 1996. Small peptides as potent mimetics of the protein hormone erythropoietin. Science 273: 458-64
46 Burns S, Arcasoy MO, Li L, Kurian E, Selander K, Emanuel PD, Harris KW. 2002. Purification and characterization of the yeast-expressed erythropoietin mutant Epo (R103A), a specific inhibitor of human primary hematopoietic cell erythropoiesis. Blood 99: 4400-5
47 Zhang H, Wilson IA, Lerner RA. 2012. Selection of antibodies that regulate phenotype from intracellular combinatorial antibody libraries. Proc Natl Acad Sci U S A 109: 15728-33
48 Cwirla SE, Balasubramanian P, Duffin DJ, Wagstrom CR, Gates CM, Singer SC, Davis AM, Tansik RL, Mattheakis LC, Boytos CM, Schatz PJ, Baccanari DP, Wrighton NC, Barrett RW, Dower WJ. 1997. Peptide agonist of the thrombopoietin receptor as potent as the natural cytokine. Science 276: 1696-9
49 Nakano K, Kojima T, Kasutani K, Senoh C, Natori O, Ishii S, Tsunoda H, Hattori K. 2009. Effective screening method of agonistic diabodies based on autocrine growth. J Immunol Methods 347: 31-5
50 Zhang H, Yea K, Xie J, Ruiz D, Wilson IA, Lerner RA. 2013. Selecting agonists from single cells infected with combinatorial antibody libraries. Chem Biol 20: 734-41
51 Iversen PO, To LB, Lopez AF. 1996. Apoptosis of hemopoietic cells by the human granulocyte-macrophage colony-stimulating factor mutant E21R. Proc Natl Acad Sci U S A 93: 2785-9
52 Reddy CC, Niyogi SK, Wells A, Wiley HS, Lauffenburger DA. 1996. Engineering epidermal growth factor for enhanced mitogenic potency. Nat Biotechnol 14: 1696-9
53 Coco WM, Encell LP, Levinson WE, Crist MJ, Loomis AK, Licato LL, Arensdorf JJ, Sica N, Pienkos PT, Monticello DJ. 2002. Growth factor engineering by degenerate homoduplex gene family recombination. Nat Biotechnol 20: 1246-50
54 Cochran JR, Kim YS, Lippow SM, Rao B, Wittrup KD. 2006. Improved mutants from directed evolution are biased to orthologous substitutions. Protein Eng Des Sel 19: 245-53
55 Lahti JL, Lui BH, Beck SE, Lee SS, Ly DP, Longaker MT, Yang GP, Cochran JR. 2011. Engineered epidermal growth factor mutants with faster binding on-rates correlate with enhanced receptor activation. FEBS Lett 585: 1135-9
56 Lui BH, Cochran JR, Swartz JR. 2011. Discovery of improved EGF agonists using a novel in vitro screening platform. J Mol Biol 413: 406-15
57 Jones DS, 2nd, Tsai PC, Cochran JR. 2011. Engineering hepatocyte growth factor fragments with high stability and activity as Met receptor agonists and antagonists. *Proc Natl Acad Sci U S A* 108: 13035-40
58 Jaszberenyi M, Schally AV, Block NL, Zarandi M, Cai RZ, Vidaurre I, Szalontay L, Jayakumar AR, Rick FG. 2013. Suppression of the proliferation of human U-87 MG glioblastoma cells by new antagonists of growth hormone-releasing hormone in vivo and in vitro. Target Oncol 8: 281-90
59 Ortega S, Schaeffer MT, Soderman D, DiSalvo J, Linemeyer DL, Gimenez-Gallego G, Thomas KA. 1991. Conversion of cysteine to serine residues alters the activity, stability, and heparin dependence of acidic fibroblast growth factor. J Biol Chem 266: 5842-6
60 Arakawa T, Horan TP, Narhi LO, Rees DC, Schiffer SG, Holst PL, Prestrelski SJ, Tsai LB, Fox GM. 1993. Production and characterization of an analog of acidic fibroblast growth factor with enhanced stability and biological activity. Protein Eng 6: 541-6
61 Dubey VK, Lee J, Somasundaram T, Blaber S, Blaber M. 2007. Spackling the crack: stabilizing human fibroblast growth factor-1 by targeting the N and C terminus beta-strand interactions. J Mol Biol 371: 256-68
62 Hsu E, Osslund T, Nybo R, Chen BL, Kenney WC, Morris CF, Arakawa T, Narhi LO. 2006. Enhanced stability of recombinant keratinocyte growth factor by mutagenesis. Protein Eng Des Sel 19: 147-53
63 Dubaquie Y, Mortensen DL, Intintoli A, Hogue DA, Nakamura G, Rancatore P, Lester P, Sadick MD, Filvaroff E, Fielder PJ, Lowman HB. 2001. Binding protein-3-selective insulin-like growth factor I variants: engineering, biodistributions, and clearance. Endocrinology 142: 165-73
64 Mercola M, Deininger PL, Shamah SM, Porter J, Wang CY, Stiles CD. 1990. Dominant-negative mutants of a platelet-derived growth factor gene. Genes Dev 4: 2333-41
65 Shamah SM, Stiles CD, Guha A. 1993. Dominant-negative mutants of platelet-derived growth factor revert the transformed phenotype of human astrocytoma cells. Mol Cell Biol 13: 7203-12
66 Vassbotn FS, Andersson M, Westermark B, Heldin CH, Ostman A. 1993. Reversion of autocrine transformation by a dominant negative platelet-derived growth factor mutant. Mol Cell Biol 13: 4066-76
67 Lin H, Chen B, Sun W, Zhao W, Zhao Y, Dai J. 2006. The effect of collagen-targeting platelet-derived growth factor on cellularization and vascularization of collagen scaffolds. Biomaterials 27: 5708-14
68 Su L, Chen S, Yang K, Liu C, Liang Z. 2006. Cloning and expression of human stem cell factor fused with thrombopoietin mimetic peptide in Escherichia coli. Biotechnol Lett 28: 857-62
69 Hsu YR, Wu GM, Mendiaz EA, Syed R, Wypych J, Toso R, Mann MB, Boone TC, Narhi LO, Lu HS, Langley KE. 1997. The majority of stem cell factor exists as monomer under physiological conditions. Implications for dimerization mediating biological activity. J Biol Chem 272: 6406-15
70 Chen T, Yang J, Wang Y, Zhan C, Zang Y, Qin J. 2005. Design of recombinant stem cell factor-macrophage colony stimulating factor fusion proteins and their biological activity in vitro. J Comput Aided Mol Des 19: 319-28