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13. ABSTRACT (<i>Maximum 200 Words</i>) <p>Macromolecular drug carriers provide one of the most promising approaches to improve delivery of therapeutic and diagnostic drugs to cancer cells. A very significant achievement in this area relates to the recent development of "long-circulating" macromolecular and colloidal preparations (polymers, micelles, liposomes, etc.). Clearance of these compounds from the blood is very slow and, as a consequence, they circulate long enough to extravasate into tumors via "leaky" endothelium, and accumulate at these sites as a result of non-specific tissue binding and spontaneous endocytosis.</p> <p>Although long circulating drug carriers do accumulate in solid tumors, they do not specifically bind cancer cells. Also, they can partially return to the blood stream by a reverse of the extravasation process through the leaky endothelium. To overcome these limitations, substantial improvements could be achieved by the association of long-circulating drug carriers with "vector molecules" capable of binding specifically to cancer cells. Our work has been based on the use of a genetic selection/screening technique to identify peptides that selectively recognize breast cancer cells (as opposed to normal cells of the same or different type). Such cancer-specific peptides were then coupled to fleximer-based drug carriers. Concomitantly, model drug carriers with cooperative tumor-targeting molecules have been developed.</p>				
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INTRODUCTION

Macromolecular drug carriers provide one of the most promising approaches to improve delivery of therapeutic and diagnostic drugs to cancer cells. A very significant achievement in this area relates to the recent development of "long-circulating" macromolecular and colloidal preparations (polymers, micelles, liposomes, etc.). These compounds are sterically protected by brushes of elongated molecules, composed, most often, of polyethylene glycol (PEG), and, for this reason, characterized by low interactions with the biological milieu. Clearance of these compounds from the blood stream and uptake by the reticuloendothelial system (RES) are very slow. As a consequence, macromolecular drug carriers circulate long enough to extravasate into tumors via "leaky" endothelium, and accumulate at these sites as a result of deficient lymphatic drainage, non-specific tissue binding and spontaneous endocytosis.

We have developed and successfully tested several long-circulating macromolecular drug carriers for systemic drug delivery to solid tumors and lymph nodes. Efficient intravenous delivery of both diagnostic and therapeutic drugs to mammary adenocarcinoma tissue and to draining lymph nodes have been demonstrated. Recently, we have further improved the technology by developing an entirely novel class of macromolecular drug carriers, which are based on a new long-circulating polymer ("Fleximer"). Fleximer-based carriers differ from the previous PEG-based derivatives in the following remarkable features: (a) fleximer structure partially emulates natural protective oligosaccharides and proteoglycans (molecular brushes), so both carrier recognition by RES and non-specific binding are very low; (b) fleximers are non-toxic and (unlike PEG) readily biodegradable; (c) While PEG derivatives can be modified only at their terminal groups, fleximer molecules can be modified throughout their main chain, thus allowing the synthesis of drug carriers with much broader variety of structures.

Although long circulating drug carriers do accumulate in solid tumors, they do not specifically bind cancer cells. Also, they can partially return to the blood stream by a reverse of the extravasation process through the leaky endothelium. These limitations have two major consequences: 1) therapeutic drug delivery has limited efficacy, so that the therapeutic index remains relatively low; 2) diagnostic imaging agents for tumor staging have low specificity and, for small metastases, low sensitivity. Substantial improvements could be achieved by the association of long circulating drug carriers with "vector molecules" capable of binding specifically to cancer cells. These molecules would (a) direct carrier binding specifically to cancer cells, minimizing nonspecific uptake by the surrounding tissue; and (b) prevent the carrier from exiting the tumor, and thus maintaining higher local drug concentrations. Previous attempts to develop cancer-specific vector molecules were based on antibodies against tumor-specific antigens. This approach, however, has several limitations, related to the large size of the antibody

molecules, and the fact that cancer-specific antigens are usually expressed at low concentrations, and only in limited subsets of cancer cells.

Our proposal was based on the idea of using a genetic selection/screening technique to identify peptides that selectively recognize breast cancer cells (as opposed to normal cells of the same or different type). Such cancer-specific peptides will then be coupled to fleximer-based drug carriers to confer upon these compounds the desired specificity. In particular, peptides were to be identified that cause selective direct association of the extra-vasated fraction of the drug carrier(s) to the cancer cells present in the tumor tissue. The peptides were to be selected in such a way that they (a) recognize a large number of independently derived breast carcinoma cells, and (b) do not interact with normal endothelium and blood components, and, therefore, not compromise drug circulation and extravasation in tumor tissues. As a result, the therapeutic index of chemotherapeutic drugs should be drastically improved, and the signal-to-noise ratio of diagnostic preparations increased.

The hope driving our work was that identification of tumorotropic peptides would significantly facilitate the development of macromolecular drugs for therapy and early diagnosis of breast cancer. To our knowledge, such an approach had not been attempted before, and had a great potential of leading to new, very powerful tools for the eradication of breast cancer.

BODY

1) Isolation of tumorotropic peptides.

One frequent genetic alteration connected with breast cancer development is amplification and/or overexpression of the Erb2 oncogene. In order to optimize our selection/screening procedure for breast-cancer specific peptides, we decided at first to focus on the identification of peptides that can selectively bind to the extracellular portion of the Erb2 protein. We were prompted by several reasons. First, by knowing what the specific target of the selected peptides would be, it would be possible to measure affinity of binding of the peptide-expressing phage, as well as of the biochemically synthesized peptide, either free in solution or coupled to a macromolecular carrier. This in turn would have allowed us to optimize conditions for additional studies with peptides binding to unidentified targets. Second, we made a collaborative arrangement with Dr. Kermit Carraway at Harvard Medical School, who provided us with SF9 insect cells overexpressing the Erb2 protein in a functional state on their surface. Use of normal SF9 cells for control experiments would eliminate all background problems due to binding of endogenous Erb2 which is normally expressed on the surface of mammalian cells.

Three phage display libraries were used for these studies and were purchased from New England Biolabs. Each of these libraries was subjected to several cycles of binding, saline washing, elution at acidic pH, and amplification using (a) Erb2-overexpressing versus control SF9 cells; (b) Erb2-overexpressing versus control NIH-3T3 cells; (c) Erb2 overexpressing versus non-expressing mammary carcinoma cell lines. The phage species recovered at the end of these cycles were cloned, purified and tested for selectivity of binding to Erb2 overexpressing versus control cells.

The first library that was used consisted of a random stretch of 7 amino acids expressed at the amino terminus of the phage gene III protein. At the end of 6 selection cycles, we analyzed 20 independent phages by nucleotide sequencing and found 12 that contained the same peptide sequence. These phages were found to bind to Erb2 overexpressing mammalian cells ~20 times more than to control cells. However, when the peptide encoded by these phages was synthesized and tested for specificity of binding, it was found to bind to a similar extent to both Erb2 overexpressing and to control cells. We also tested whether the affinity of binding of this peptide might be increased when tested in a bound form, after chemical coupling to a macromolecular carrier. However, even in this case results were essentially negative.

For this reason, we decided to abandon this peptide and look for additional ones that might have either a longer primary sequence or a constrained secondary structure. The second library that we screened consisted of phages with a random 12 amino acid sequence inserted at the amino terminus of the gene III protein as in the previous library. At the end of 6 cycles of screening, no phages were recovered that bound specifically to Erb2 overexpressing cells.

We thus switched to a third library of phages that express a 7 amino acid random sequence flanked by a cystein at both ends, and inserted at the amino terminus of the gene III protein. After only two rounds of screening, more than 80% of the phages that were examined expressed the same peptide sequence. This peptide sequence was not found in 20 random phages that were examined from the initial library. The sequence of this peptide is:

Cys-Pro-Asn-Pro-Asn-Asn-Lys-Asn-Cys

Computer simulation for the most thermodynamically-favorable configuration of the peptide pointed to a very interesting feature, namely that the peptide can be present in a cyclic form:

-Cys-Pro-Asn-Pro-Asn-Asn-Lys-Asn-Cys-
|-----|

which can be dramatically stabilized when complexed with calcium ion (Figure 1). This finding raised the possibility that the peptide, either in its phage-bound or chemically synthesized form, binds to Erb2-expressing cells in a calcium-dependent manner. However, no such calcium-dependence was observed. Furthermore, the peptide expressed on the phage surface exhibited only a 4-7 fold increased specificity of binding on Erb2 expressing cells versus controls, and no such specificity was observed when the peptide was tested in a free soluble form or as a complex with a macromolecular carrier.

For this reason, we decided to abandon this peptide and look for an improved screening strategy that might enhance our chances of success. In particular, the main problem that we encountered in all our screening attempts, was the isolation of only a very limited number of phages expressing Erb2-binding peptides. Our priority at this point was that of isolating a much higher number of phages different expressing Erb2-binding peptides, so that we would have greater chances of identifying peptides that would maintain high affinity and specificity of binding even when tested after chemical synthesis. Thus, we devoted our subsequent efforts to the optimization of a phage panning selection using as target the purified extracellular domain of the ERb2 protein. Dr. Carraway was recently able to produce this domain of Erb2 in SF9 cells as a secreted soluble, form. The protein produced in this manner appears to retain much of its native configuration, as indicated by the fact that it is still recognized by antibodies that recognize extracellular Erb2 only under native conditions. Panning of phage libraries on the purified Erb2 instead of Erb2-expressing cells would have the significant advantage of eliminating the binding of phage to other membrane cellular proteins, and therefore should greatly increase our chances of obtaining a much larger collection of Erb2-binding phages. In our subsequent studies, we performed phage panning experiments with Petri dishes coated with the Erb2 protein. A blocking solution with BSA was used to prevent aspecific binding to plastic. However, these blocking conditions were too strong and need to be better optimized. Alternatively, the soluble Erb2 protein is produced as a his-tagged form, so that it will be possible to perform phage panning selection with the protein attached to beads rather than absorbed to dishes.

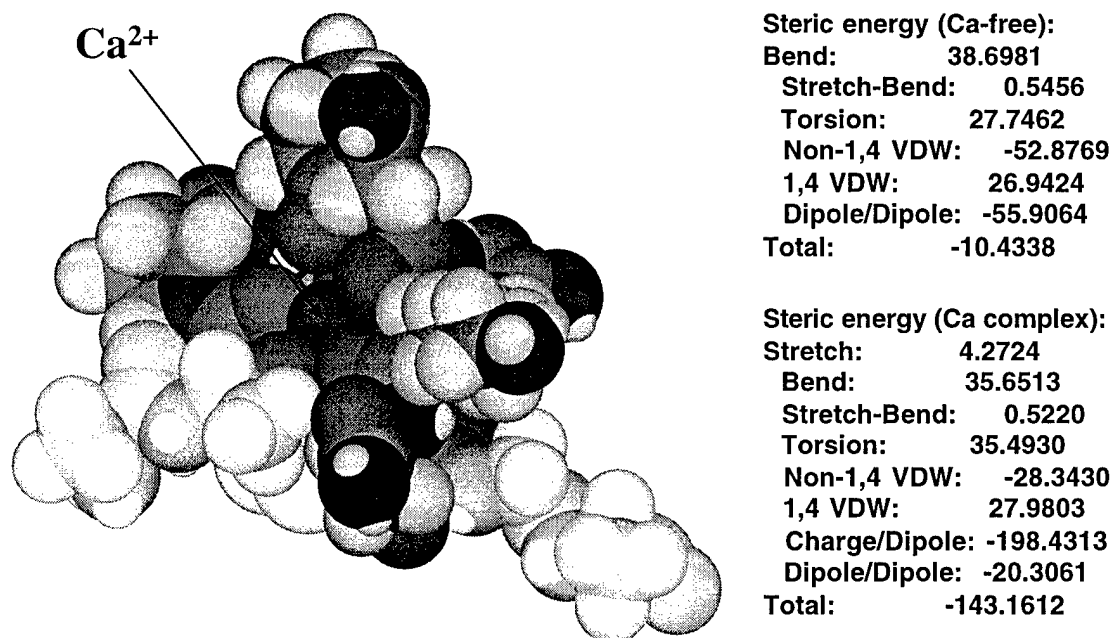


Figure 1. Cyclic form of Cys-Pro-Asn-Pro-Asn-Asn-Lys-Asn-Cys forms a potential metal-binding site (molecular simulation). Steric energies calculated based on MM2 model.

2) Development of model drug carriers with cooperative tumor-targeting molecules.

While testing phage display libraries for breast cancer-targeted peptides, we continued developing prototype cooperative carrier systems utilizing a previously developed model system based on N-formylpeptide - formylpeptide receptor interaction.

Peptides binding membrane markers of white blood cells, including the leukocyte N-formylpeptide receptor (FPR), have been found to accumulate in inflammatory regions as a result of association with white blood cells invading the inflammation site. This fact was utilized to study the behavior of cooperative polymer-based vectors comprising several peptide moieties, as compared to the analogous monomeric peptide. The objectives of this fragment of the study were: (i) prepare macromolecular preparations of varying length and comprising varying number of chemotactic peptide (N-formyl-Met-Leu-Phe-Lys) moieties per macromolecule length; (ii) determine the optimal range of peptide content; (iii) determine the molecule size range providing a most favorable blood clearance rate for agent accumulation in inflammatory areas; (iv) synthesize a model agent for inflammation scintigraphy comprising a g-emitting radionucleotide and a fluorescent label; (v) characterize the biokinetics and imaging characteristics of the model preparation as compared to the radiolabeled peptide monomer.

Macromolecules comprising multiple f-Met-Leu-Phe-Lys (fMLFK) moieties and DTPA were synthesized using linear poly[hydroxymethyl-ethylene hydroxy-methyl-formal] (PHF) backbone. PHF, a biomimetic “stealth” polymer, is a base compound of Fleximer family. An aldehyde form of PHF with broad molecular weight distribution (10 to 150 kDa) was conjugated with cystamine (spacer precursor) and fMLFK in one stage, at various molar ratios, in the presence of cyanoborohydride. The conjugates were subsequently reduced with borohydride to transform the unused aldehyde into hydroxymethyl groups and S-S bridges to mercaptogroups. The latter were derivatized with DTPA anhydride and (in some preparations) with trace amounts of fluorescein-5-maleimide. The resultant polymers were purified by gel chromatography, fractionated by SEC HPLC, and labeled with ^{111}In via transchelation in citrate buffer. Control polymers containing DTPA but no peptide were prepared analogously. Radiolabeled peptide monomer was prepared via fMLFK derivatization with DTPA and labeling with ^{111}In .

Pilot biokinetics and biodistribution studies were performed in normal male CD rats ($n=3$ per preparation) to determine the optimal ranges of peptide content and molecular weight of the polymer. Conjugates with broad molecular weight distribution were fractionated by SEC HPLC to obtain fractions with MW=10, 50 and 80 kDa (MW distributions overlapped by ca. 30% by HPLC). For the preliminary testing, conjugates containing from 0.5 to 20 % fMLFK (mol/mol monomer) were prepared. Pilot studies showed that the optimal range by conjugate content is ca. 3-10%, and the optimal dose range, with regard to clearance rate, is > 0.1 mg/kg.

For the biodistribution studies in rabbit inflammation model, preparations containing 5% of each fMLFK and DTPA were synthesized. Non-overlapping 15 and 75 kDa fractions used in this experiment showed statistically significant difference in biokinetics. The preparations were labeled with ^{111}In and injected iv into 2.5 kg New Zealand rabbits, $n=4$ per group, 50-100 $\mu\text{Ci}/\text{animal}$ (0.5 mg/kg total substance). Animals were normal or bearing focal bacterial inflammation induced by inoculation of E.Coli (clinical isolate) in thigh muscle. Indium-labeled PHF-DTPA and monomeric DTPA-fMLFK were used as control preparations. Images were acquired over a 20 hr. period, followed by a biodistribution study. Biodistribution data showed that, compared to the monomer DTPA-fMLFK, renal accumulation was greatly reduced (by 81% and 88% for 70 and 15 kDa preparations respectively), while accumulation in the infected muscle was reduced by only 37% and 72%. Target to normal muscle ratios were 25 ± 10 and 14 ± 4 , compared to 33 ± 21 for the monomer. In blood, testes, adrenals and heart, accumulation was reduced by 40-50%. Hepatic and splenic depositions were reduced by 40% for the 15 kDa preparation, and increased by 50% for the 70 kDa one. In other tissues, label content

did not significantly differ from the monomer. By 20 hr., accumulation of DTPA-PHF-fMLFK in the infected site resulted in clear delineation of the inflammation in all images. It has been found that the high molecular weight preparation accumulate in the inflammation, in part, as a result of non-specific vascular leakage/retention, analogous to that in tumors. Accumulation of the low molecular weight preparation in the inflammatory site was completely target-specific, i.e., entirely dependent on the action of the chemotactic peptide. Based on this observation, we hypothesize that small targeted polymers may be preferable as targeted carriers; this hypothesis will be further tested in future research.

Key Research Accomplishments

1) We have optimized conditions and identified peptides that selectively bind to Erb2, an important cell surface receptor overexpressed in breast cancer cells, when expressed on the surface of a filamentous phage particle. However, unsatisfactory results were obtained when the same peptides were tested in solution and/or conjugated to macromolecular drug carriers.

2) We have developed model macromolecules bearing multiple fMLFK which exhibited dramatically reduced renal accumulation, practically unchanged deposition in RES (which has been a major concern regarding preparations of this type), and excellent accumulation in the target, thus demonstrating the potential benefits of the approach.

Reportable outcomes

Papisov MI, Babich JW, Dotto P, Barzana M, Hillier S, Graham-Coco W, Fischman AJ. Model cooperative (multivalent) vectors for drug targeting. 25th Int. Symp. on Controlled Release of Bioactive Materials, 1998, Las Vegas, Nevada, USA; Controlled Release Society, Deerfield, IL, 1998; 170-171.

Papisov MI, Omelyanenko V, Terez I, Myagchilov S. Dynamic modeling of protective molecular brushes. 25th Int. Symp. on Controlled Release of Bioactive Materials, 1998, Las Vegas, Nevada, USA; Controlled Release Society, Deerfield, IL, 1998; 242-243.

Papisov MI. Surface protection in bioengineering. Abstracts of The Whitaker Foundation Biomedical Engineering Conference, August 1999, La Jolla, Ca. The Whitaker Foundation, VA, 1999,49.

Conclusions

We have optimized conditions and screened three different phage display libraries for peptides that selectively bind to Erb2, an important cell surface receptor overexpressed in breast cancer cells. The first peptide sequence that was identified on an Erb2 binding phage did not retain the same specificity of binding when tested as an isolated peptide or a peptide bound to a macromolecular drug carrier. A second Erb2 binding peptide with a constrained secondary structure has also been identified on a phage, and also had low binding affinity. Thus, the phage display fragment of this study illustrated the drawbacks of the method, and reiterated the need for binding affinity amplification. On the other hand, the model macromolecules bearing multiple fMLFK demonstrated dramatically reduced renal accumulation, practically unchanged deposition in RES (which has been a major concern regarding preparations of this type), and excellent accumulation in the target, which demonstrates the potential benefits of the approach. Optimization of macromolecule size and peptide composition/content can be expected to provide, in this particular case, preparations with superb targeting capabilities. However, developing such preparations will require detailed investigation of the binding properties of the vector peptides (which was not the scope of this study).

We have confirmed that the phage display technology can be used to identify peptides that bind to proteins expressed on the surface of breast cancer cells. The challenge remains to identify peptides that retain specificity of binding when separated from the phage. In our ongoing study, we will investigate binding properties of Erb2-targeted peptides in detail, measuring fluorescence anisotropy lifetimes to determine binding kinetics and binding energies.

Model technologies for producing cooperative (multivalent) vectors for drug targeting utilizing multiple peptide moieties has been demonstrated. The benefits of macromolecule size and peptide content optimization has also been demonstrated. Compact, sterically protected drug carriers capable of accommodating (a) multiple peptide moieties on the surface and (b) drug in the inner core can be synthesized as graft copolymer-like constructs. Their cooperative interaction with target cells may depend on the mobility of side chains. The latter can be optimized, which is a subject of our current studies.

References

- Gallop, MA, Barrett, RW, Dower, WJ, Fodor, SPA, and Gordon, EM (1994). Applications of combinatorial technologies to drug discovery. 1. Background and peptide combinatorial libraries. *J. Med. Chem.*, 37, 1233-1251.
- Cassidy, J., Newell, D.R., Wedge, S.R., and Cummings, J. (1993) Pharmacokinetics of high molecular weight agents. *Cancer Surveys*, 17, 315-341.
- Kotani, M., Matsuno, K., Ezaki, T., and Ueda, M. (1980). The increase in permeability of postcapillary venules in lymph nodes subjected to the regional graft-versus-host reaction. *Lab. Investigation* 42, 589-595
- Frank, M.M., and Fries, L.F. The role of complement in inflammation and phagocytosis (1991). *Immunology today*, 12, 322-326.
- Royall, J.A., Berkow, R.L., Beckman, J.S., Cunningham, M.K., Matalon, S., and Freeman, B.A. (1989) Tumor necrosis factor and interleukin 1 alpha increase vascular endothelial permeability. *Am. J. Physiol.* 257 L399-L410.
- Fujita, S, Puri, R., Yu, Z.-X., Travis, W., Ferrans, V. (1991) An ultrastructural study of in vivo interactions between lymphocytes and endothelial cells in the pathogenesis of the vascular leak syndrome induced by interleukin-2. *Cancer*, 68, 2169-2174.
- Jain RK. Transport of molecules across tumor vasculature. *Cancer & Metastasis Reviews*. 6(4):559-93, 1987.
- Gregoriadis, G. (Ed) *Liposome Technology*, 2nd Edn., CRC Press, Boca Raton, 1993.
- Woodle MC. Sterically stabilized liposome therapeutics. *Adv. Drug Delivery Rev.* 1995, 16:249-266
- Gref R, Domb A, Quelec P, Blunk P, Muller RH, Verbavatz JM, Langer R. The controlled intravenous delivery of drugs using PEG-coated sterically stabilized nanospheres. *Adv. Drug Delivery Rev.* 1995, 16:215-234
- Papisov MI. Modeling in vivo transfer of long-circulating polymers (two classes of long circulating polymers and factors affecting their transfer in vivo). *Adv. Drug Delivery Rev.*, Special issue on long circulating drugs and drug carriers, 1995, 16:127-137.

Papisov MI, Weissleder R, Brady TJ. Diagnostic targeting of lymph nodes with polymeric imaging agents. In: Torchilin VP, ed. Targeted delivery of imaging agents. CRC Press, Boca Raton, 1995: 385-402

Papisov MI, Weissleder R, Schaffer B, Bogdanov AA, Brady TJ. Intravenous carriers for drug delivery to lymph nodes. In: Sixth International Symposium on Recent Advances in Drug Delivery Systems, Salt Lake City, UT. University of Utah, 1993; 137-138.

Papisov MI, Garrido L, Poss K, Wright C, Weissleder R, Brady TJ. A long-circulating polymer with hydrolyzable main chain. In: 23 International Symposium on Controlled Release of Bioactive Materials, Kyoto 1996, CRS 1996, in press.

Papisov MI, Poss K., Weissleder R., and Brady TJ. Effect of steric protection in lymphotropic graft copolymers. In: Seventh international symposium on recent advances in drug delivery systems, Abstracts; Salt Lake City, UT, 1995; University of Utah 1995, 171-172

Papisov MI, Martin C, Poss K., Weissleder R., and Brady TJ. Amplification of steric effects in cooperative systems. In: 22-nd International Symposium on Controlled Release of Bioactive Materials, 1995, 442-443.

Torchilin VP, Omelyanenko V, Papisov MI, Bogdanov AA, Trubetskoy VS, Herron DIN, Gentry CA. Poly(ethyleneglycol) on the liposome surface: on the mechanism of polymer-coated liposome longevity. BBA 1994, 1195:11-20.

Poss K, Bogdanova AV, Weissleder R., Brady TJ, Papisov MI. Cytotoxicity of doxorubicin covalently linked with the backbone of lymphotropic graft copolymers. In: Seventh international symposium on recent advances in drug delivery systems, Abstracts; Salt Lake City, UT, 1995; University of Utah 1995, 173-174

Harika L, Weissleder R, Poss K, Papisov M. A macromolecular intravenous agent for MR lymphography: characterization and efficacy studies. Radiology 198:365-370, 1996

Papisov MI, Savelyev VY, Sergienko VB, Torchilin VP. Magnetic drug targeting (I) In vivo kinetics of radiolabeled magnetic drug carriers. Int J Pharm 1987; 40:201-6.

Goodson, RJ, Doyle, MV, Kaufman, SE, and Rosenberg, S (1994). High-affinity urokinase receptor antagonists identified with bacteriophage peptide display. Proc. Natl.

Acad. Sci. USA 91, 7129-7133.

Yayon, A., Aviezer, D., Safran, M., Gross, J.L., Heldman, Y., Cabilly, S., Givol, D. and Katchalski-Katzir, E. (1993). Isolation of peptides that inhibit binding of basic fibroblast growth factor to its receptor from a random phage-epitope library. Proc. Natl. Acad. Sci. USA 90, 10643-10647.

Ealovega, M.W., McGinnis, P.K., Sumantran, V.N., Clarke, M.F. and Wicha, M.S. (1996). *bcl-x_s* gene therapy apoptosis of human mammary tumors in nude mice¹. Cancer Research 56, 1965-1969.

Fink, LM and Hsu, P-L (1994). Bacteriophage epitope libraries. Virchows Archiv. 424, 1-6.

Smith, G.P. and Scott, J.K. (1993) by Academic Press Inc. Libraries of peptides and proteins displayed on filamentous phage. Meth. Enzy. 217, 228-257.

Koivunen, E., Wang, B., Dickinson, C.D. and Ruoslahti, E. (1994) by Academic Press Inc. Peptides in cell adhesion research. Meth Enzy. 245, 346-369.

Folgori, A., Tafi, R., Meola, A., Felici, F., Galfre, G., Cortese, R., Monaci, P. and Nicosia, A. (1994). A general strategy to identify mimotopes of pathological antigens using only random peptide libraries and human sera. EMBO J. 13, 2236-2243.

Yayon, A., Aviezer, D., Safran, M., Gross, J.L., Heldman, Y., Cabilly, S., Givol, D. and Katchalski-Katzir, E. (1993). Isolation of peptides that inhibit binding of basic fibroblast growth factor to its receptor from a random phage-epitope library. Proc. Natl. Acad. Sci. USA 90, 10643-10647.

Pascualini, R. and Ruoslahti, E. (1996). Organ Targeting in vivo using phage display peptide libraries. Nature, 380:364-366

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