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ARTICLES

Applications of One-Bead One-Compound Combinatorial Libraries and Chemical Microarrays in Signal Transduction Research

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ABSTRACT

The "one-bead-one-compound" (OBOC) combinatorial library method synthesizes millions of random compounds such that each bead displays only one compound. Bead libraries are screened, and positive beads are isolated for structure analysis. Peptide substrates and inhibitors of protein kinases, and peptide ligands for cell surface receptors have been identified using this method. A novel encoding strategy for OBOC libraries has been developed to identify peptidomimetic and small-molecule ligands that specifically interact with cellular proteins. These ligands will be tested for their effects on cell signaling and used to construct chemical microarrays for further characterization of ligand−protein interactions.

Introduction

Many signaling pathways begin when a cell encounters an external stimulus at the cell surface. These signaling cascades transmit signals through the membrane and into the cytoplasm of the cells, which triggers specific gene transcription and other cellular responses. Combinatorial chemistry is an enabling technology that can be used to study events occurring in the signaling pathways.[1] In combinatorial chemistry, a large number of compounds can be generated and screened for specific biological or biochemical function. Such compounds may also exert interesting biological or biochemical effects on intact cells. These compounds may have therapeutic value as well. In the last seven years, we have applied the OBOC combinatorial library method [2,3] to identify cell surface ligands, determine peptide substrate motifs for protein kinases, and develop pseudosubstrates and small-molecule inhibitors for protein kinases. Although peptides are useful for targeting extracellular macromolecules or cell surface receptors, they are generally not permeable to cell membranes and cannot target intracellular proteins. To help us find peptidomimetics or small-molecule compounds that will more easily enter the cell so that we can probe the activities of intracellular signaling proteins, we have developed a novel encoding strategy for OBOC peptidomimetic and small-molecule libraries.[4] Peptidomimetic beads or small-molecule beads that interact with specific intracellular proteins can therefore be isolated and identified. In addition, to efficiently characterize the interactions between the leads and the target molecules, we have developed several chemical microarray methods that use only minute quantity of analytes and are highly efficient and miniaturized. In this article, we shall give a brief account of these technologies, followed by a discussion on the application of these technologies to study signal transduction.

OBOC Combinatorial Peptide Libraries

The five general combinatorial peptide library methods include (i) biological library methods such as phage-display,[5] (ii) spatially addressable libraries such as multipin technology,[6] SPOT synthesis method,[7] and peptide microarray techniques,[8,9] (iii) mixture libraries that require deconvolution such as the positional scanning method,[10] (iv) the OBOC combinatorial library method,[2,3] and (v) solution peptide libraries with affinity chromatography.[11]

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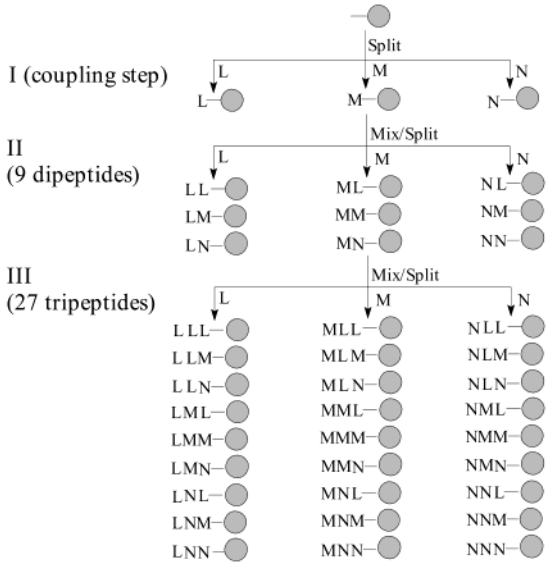

FIGURE 1. Synthetic scheme of the "split-mix synthesis" method to generate a one-bead one-compound combinatorial library.

Each of these methods has advantages and disadvantages, with the choice of the method greatly dependent on the nature of the biological targets in question, the reagents available for screening, the expertise of the investigators, and the resources available. For detail description on this subject, please refer to our recent reviews.[1,12]

In 1991, we first reported the development and application of the OBOC combinatorial library method to identify peptide ligands against anti-β-endorphin monoclonal antibody and streptavidin.[2] In this method, millions of amino-derivatized beads (e.g., TentaGel S resin, Rapp Polymere, Tubingen, Germany) are used as the solid support and after several rounds of "split-mix" synthesis (Figure 1),[2,13,14] with each bead displaying only one peptide entity. There are approximately 100 pmole or 10^{13} copies of the same peptide on each of these 90 μm beads. The OBOC library method is extremely efficient, since thousands to millions of peptides or small molecules can be synthesized within a week. We generally use standard Fmoc chemistry to prepare peptide libraries. In addition to standard eukaryotic amino acids, unnatural amino acids[15] and D-amino acids are often included in our library construction. The bead library is then screened for a specific biological property using a number of different assays we have developed over the past decade to screen OBOC libraries.[3] Positive beads are physically isolated for structural analysis. For peptide libraries that consist of α-amino acids, we routinely use an automatic protein sequencer (e.g., ABI 494) to determine the amino acid sequence of the positive beads.[15] Specific screening assays and their applications for signal transduction research will be discussed below.

Encoded Peptidomimetic and Small-Molecule Combinatorial Libraries

While peptide beads can be reliably sequenced by an automatic protein microsequencer, structural determination of the peptidomimetic or small-molecule compound in one single bead is challenging. Unsuccessful attempts have been made to use spectroscopic methods such as mass spectrometry, nuclear magnetic resonance, and infrared spectroscopy to directly determine the complete chemical structure of an unknown compound contained in a single 80−100 μm bead isolated from large diverse libraries (e.g., 100 000 compounds). This is primarily due to the lack of consistent fragmentation pattern or sensitivities in some of these methods. Over the past decade, a number of different encoding methods (chemical and physical encoding) have been developed to achieve this task (for reviews, see refs 3 and 16). The more successful approach is the chemical encoding method in which each building block used in the synthesis of the small-molecule library is encoded by a coding block such as fluorophenyl ether,[17] peptide,[18] secondary amine, or trityl-based mass tag. However, these methods suffer two disadvantages. First, the encoding process is slow because it requires the orthogonal coupling of the building block and the coding block. Second, the encoding molecules may interfere with the testing molecules during the screening process. To solve these two problems, we have recently developed a novel peptide-encoding strategy such that the testing molecule is on the bead surface and the peptide coding tag is located within the interior of the bead.[4] This encoding method is highly efficient as each of the building blocks is incorporated into the testing arm (bead surface) and the coding peptide backbone (bead interior) simultaneously. Consequently, no additional synthetic steps are needed. After screening, the positive beads are isolated and the peptide coding tags containing α-amino acids, which have side chains derivatized with the building blocks, decoded by Edman sequencing. Alternatively, the peptide code can be cleaved off the resin bead and the cleaved product analyzed by mass spectrometry. In our original report, an encoded library of peptidomimetics with three points of randomization (over 150 000 combinations of ligands) was synthesized and screening identified high affinity peptidomimetic ligands for streptavidin.[4] We are now using this method to identify peptidomimetic and small-molecule ligands that bind to intracellular signaling proteins (see below).

Chemical and Peptide Microarray

We have successfully used the OBOC combinatorial library approach to identify ligands for a number of macromolecular targets and substrates for several protein kinases. To confirm the binding or substrate specificities of these peptides, peptides are often resynthesized on beads using a parallel synthesis method. Samples from each of these peptide beads are then tested for binding specificities. However, this resynthesis and retesting step is laborious. Therefore, a high-throughput chemical microarray system has been developed so that initial leads can be profiled efficiently. We use an automatic arrayer, which is commonly used for DNA microarrays technology to spot peptide or chemical microarrays. Because short peptides or small molecules usually do not readily adsorb to glass

surfaces, a covalent attachment method for ligand immobilization has been developed.[19] The ligands are first synthesized on Rink resin, cleaved, purified, and then spotted onto the glass surface with an automatic arrayer. A chemoselective ligation method was selected so that the free amino or sulfhydryl groups on the ligand will not be affected.[20] In this method, the glass surface is first derivatized with a glyoxylyl group, and the ligand to be spotted is attached to an oxy-amino group via a hydrophilic linker. Upon spotting, the oxy-amino group reacts with the glyoxylyl group to form an oxime bond. In a second method, a polystyrene slide is first coated with neutravidin and the biotinylated ligands are spotted on the slide. In a third method, the ligands are chemoselectively ligated to bovine serum albumin, and the peptide−protein conjugates are then spotted and adsorbed onto the polystyrene slide. Another method, used by Schreiber's group, employs the use of Michael addition to ligate thiol-containing compounds to maleimide-derivatized glass slides to form microarrays.[21] For a recent review on the subject, please refer to our recent publications.[9,20]

Protein Kinase Peptide Substrate Identification

In 1994, we reported the use of OBOC combinatorial peptide libraries to identify peptide substrate for specific protein kinases.[22] In this method, random hexa- or heptapeptide bead libraries are first generated. The middle residue of the peptide library is generally fixed as tyrosine and serine/threonine when tyrosine kinases and serine/threonine kinases are screened, respectively. The bead library (e.g., 500 000 beads) is mixed with [γ-^{32}P] ATP and a specific protein kinase. After incubation for 1 h, the bead library is thoroughly washed, heated with 0.1 M HCl for 10 min to hydrolyze the ATP, washed again, and then immobilized on a glass plate or plastic sheet with agarose. After drying overnight, the immobilized beads are exposed to an X-ray film. A day or two later, the film is developed, and the radiolabeled beads are localized and excised from the dried agarose. The collected beads then undergo a secondary screen, in which the beads are diluted with additional agarose solution, immobilized, and exposed to film. Individual radiolabeled beads can be easily retrieved for sequence analysis. Using this technique, we confirmed RRXS or RRXT as the substrate motif for cAMP-dependent protein kinase[22] and identified YIYGSFK[23] and GIYWHHF[24] as efficient substrates for p60^{c-src} protein tyrosine kinase (PTK). Because each peptide in the OBOC library is spatially separable, unique peptides with different motifs can potentially be identified. This is very different from the solution phase peptide library with affinity column selection method,[11] with which only one predominant substrate motif can be determined because the captured peptide mixture is sequenced together in one batch. When a random peptide library was screened against c-Abl PTK, 14 peptides with three distinct motifs were identified (Table 1).[25] The first motif is predominantly acidic, the second motif is also acidic and contains three or more aromatic amino acids, and the third motif is basic. All

Table 1. The Three Distinct Substrate Motifs Identified for c-Abl PTK[25]

motif I (acidic)	motif II (acidic + aromatic)	motif III (basic)
EEIYEEPY	EEIYDYAY	ERIYARTK
EEIYAEWF	EEIYYYVH	LRIYERPR
EEIYDEIY	EEIYYYEL	DVIYRRHY
DDIYEIYW	YEIYDYYV	EHIYHRHM
	YEIYDYIE	PRIYDIPY

Table 2. Comparison of Peptide Phosphorylation by Bcr-Abl and c-Abl PTK[26]

group I		group II	
peptide	phosphorylation ratio[a]	peptide	phosphorylation ratio
YEIYDYIE	2.29	DVIYRRHR	5.06
YEIYDYYV	2.91	EEIYYYVH	5.24
EEIYDYAY	3.33	PRIYDIPY	5.97
EEIYEEPY	3.38	ERIYARTK	6.79
EEIYDEIY	3.44	EHIYHRHM	8.27
EEIYYYEL	3.52	LRIYERPR	8.73
EEIYAEWF	3.98		
DDIYEIYW	4.92		

[a] Phosphorylation ratio = phosphorylation of peptide by Bcr-Abl PTK/phosphorylation of the same peptide by c-Abl PTK.

these 12 peptides are better substrates for Bcr-Abl PTK than for c-Abl PTK. However, when we compared the phosphorylation rate of each of these peptides by Bcr-Abl with that of c-Abl, two groups of peptides emerged (Table 2).[26] Group I peptides have a phosphorylation ratio (phosphorylation rate by Bcr-Abl/phosphorylation rate by c-Abl) that ranges between 2 and 5, whereas group II peptides have a phosphorylation ratio that ranges between 5 and 9. Group II peptides are predominantly basic with arginines or lysines in five of the six peptides, suggesting that basic peptides are preferred by Bcr-Abl PTK even though the primary sequence of the catalytic domain of Bcr-Abl and c-Abl is identical.[26] When we examine the amino acid sequence of the phosphorylation sites of known cellular protein substrates for c-Abl (e.g., RIN1, p62dok, CBL, Paxillin, Shc, Crk1 and BCR), we can detect some homology with the peptide substrate motifs identified from the library method.[25] Peptide substrates identified through combinatorial library methods can also be used as exogenous substrates to develop protein kinase assays.[27] They can also be printed on microarray and used for protein kinase profiling[19] (see below).

Development of Protein Kinase Inhibitors

Another interest in our laboratory is to use combinatorial chemistry to develop protein kinase inhibitors. Most of the existing PTK inhibitors compete at the ATP binding site,[28] which is not surprising since most of the lead compounds were discovered by random screening of small molecules that inhibit the kinase activity and target the deep ATP binding pocket at the catalytic site. Despite the common usage of ATP as substrates for many intracellular enzymes, some of the inhibitors discovered are very specific. Computational chemistry has also been

widely used in the discovery of small-molecule inhibitors that bind to the ATP binding site.[29] However, little work has been done on developing inhibitors that bind at other sites on the enzyme. In our laboratory, we use two approaches to discover other inhibitor leads. The first approach is to take advantage of our knowledge of the peptide substrate identified from the random peptide library and to use these substrates as templates to develop pseudosubstrate-based peptide and peptidomimetic inhibitors. The second approach is to screen diverse encoded OBOC peptidomimetic or small-molecule combinatorial libraries (up to a million diversity) with tagged purified protein kinases for binding activity.

Pseudosubstrate-Based Peptide or Peptidomimetic Inhibitors for Protein Kinases

As indicated above, we have used the OBOC combinatorial library method to discover peptide substrates for a number of different protein kinases. Using the peptide sequence YIYGSFK[23] (K_m = 55 μM) obtained by OBOC library screening as a template, we have designed and synthesized a series of conformationally and topographically constrained substrate-based peptide inhibitors for $p60^{c-src}$ PTK, with IC_{50} values in the low-micromolar range (1−3 μM).[30] We have also designed and synthesized several pseudosubstrate-based peptide inhibitors using GIYWHHY (another $p60^{c-src}$ PTK substrate with K_m = 25 μM) as a template.[24,31] Some of these peptide inhibitors are highly potent and specific with IC_{50} in the low micromolar range. Because both YIYGSFK and GIYWHHY are efficient and specific substrates for $p60^{c-src}$ PTK, chimeric branched peptides based on these two sequences have been synthesized. These branched peptides inhibit $p60^{c-src}$ PTK with high potency, indicating that the enzyme-active site of $p60^{c-src}$ PTK can accommodate more than a linear motif. This may explain why several peptides with very different linear structures can all appear to be phosphorylated by this enzyme. Additionally, Lineweaver−Burk plots of the inhibition of the $p60^{c-src}$ PTK activity by GI-(2′-Nal)WHHY or GI(2′-Nal)WHH using YIYGSFK as substrate demonstrated that these two peptides inhibit $p60^{c-src}$ PTK noncompetitively (2′-Nal, L-2-naphthylalanine), suggesting that GIYWHHY and YIYGSFK may bind to different but perhaps overlapping sites. On the basis of a weak peptide substrate MIYKYYF identified by the OBOC technique, we have identified a potent pseudosubstrate-based peptide inhibitor, CIYKYY for $p60^{c-src}$ PTK with an IC_{50} of 0.6 μM.[32]

Knowing that IY is a minimal common motif as a peptide substrate for phosphorylation by $p60^{c-src}$PTK, we designed a peptidomimetic library R−IY−X (wherein R represents 96 alkyl groups derived from 96 aldehydes via reductive alkylation and X stands for 38 amino acids that include both the L- and D-isomers of all 20 amino acids except cysteine) that was screened for phosphorylation by $p60^{c-src}$.[33] Some of these peptidomimetic beads were phosphorylated, and two of the prominent R groups were derived from 2,3-thiophenedicarboxyaldehyde and anthraldehyde. Several analogues were designed based on these peptidomimetic substrates and tested for inhibitory activity against $p60^{c-src}$PTK and found to have an IC_{50} ranging between 5 and 20 μM. Unlike the peptide inhibitors described above, these compounds showed significant growth inhibitory or morphological effects on various cancer cell lines, including v-src transfected 3T3 cells, prostate, colon, and bladder carcinoma. These compounds are excellent leads for further development.

Development of Peptidomimetic and Small-Molecule Inhibitors for Protein Kinases

As indicated above, our approach to develop peptidomimetic or small-molecule inhibitors for protein kinases is based on screening encoded peptidomimetic and small-molecule OBOC combinatorial libraries for binding activity using the previously mentioned enzyme-linked colorimetric assay.[2] In this method, a purified protein kinase is first conjugated to alkaline phosphatase and incubated with the bead libraries. The beads are thoroughly washed, and an alkaline phosphatase substrate, 5-bromo-4-chloro-3-indoyl-phosphate (BCIP), is then added. Beads that turn turquoise color are isolated for microsequencing. A variation of the above screening method is to biotinylate the protein kinase and then use a streptavidin−alkaline phosphatase conjugate to probe the beads. The identified ligands could potentially bind to any pocket or crevices on the surface of the enzyme. If one wants to eliminate ligands that bind to the ATP binding pocket, one may screen the library in the presence of high level of ATP. The ligands identified by this method will be resynthesized and tested for their inhibitory activity on in vitro protein kinase assay[27] and also on intact cancer cell lines that overexpress the protein kinase, e.g., $p60^{c-src}$ PTK. The latter assay is important because some of the ligands may inhibit the signaling pathway by blocking the interaction of the protein kinase with other associated proteins inside the cell, but may not exhibit any effect on the in vitro kinase assay. Preliminary work in our laboratory with this approach has resulted in the identification of several small-molecule inhibitors for Etk and Brk PTK.

Cell Surface Ligands Identification

Many signal transduction pathways begin when an external stimuli such as a growth factor or peptide hormone that bind to cell surface receptors. Through analysis of the human genome, many orphan membrane receptors have been identified, but their ligands and functions are yet to be determined. Even for those with known ligands, there is a need for the development of agonists or antagonists that can be used as research tools or as lead compounds for drug development. In the cancer therapeutics area, several monoclonal antibodies (Mabs) that target cell surface receptors have been approved by the FDA for cancer treatment. While cell-surface targeting Mabs are being developed into therapeutic agents, investigators are also looking into the possibility of using combinatorial library methods to identify peptides that

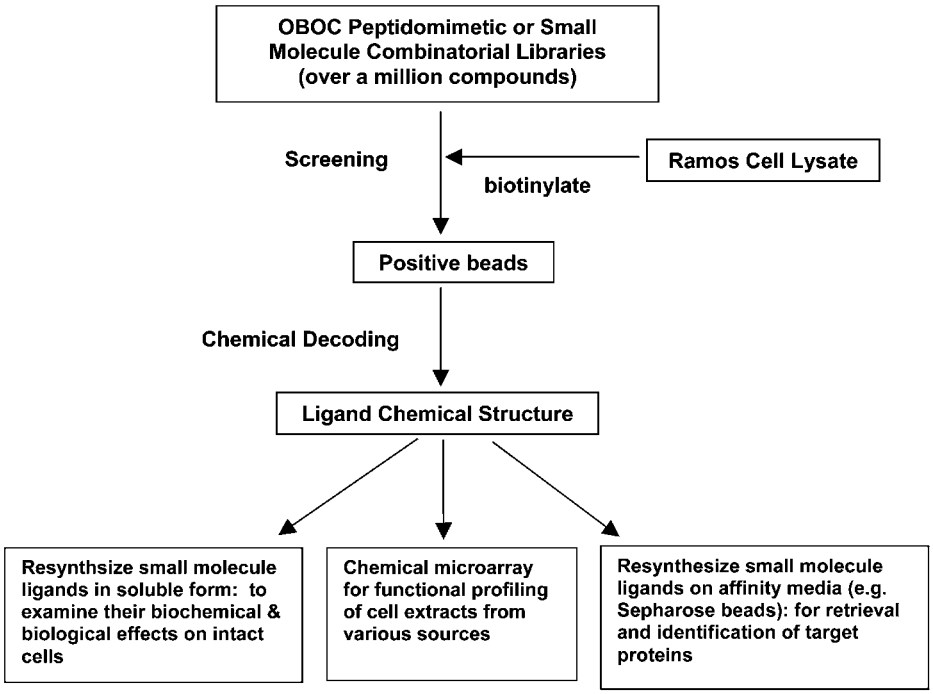

FIGURE 2. Experimental approach to functional proteomics.

target cell surface receptors. For a review on this subject, please refer to our recent publication.[34] Many investigators use the phage-display peptide library methods to identify peptides that bind to the cell surface molecules. Phage-display libraries can be screened in vitro through panning with immobilized purified proteins[35,36] or intact cells,[37] or in vivo by injecting the phages intravenously into the mouse, followed by recovering of phages from the isolated organs or tumors.

Our approach for the discovery of ligands for known cell surface receptors is to screen OBOC combinatorial bead libraries with either purified protein[38] or intact cells.[39−41] For purified soluble proteins, we usually use an enzyme-linked colorimetric assay to screen the bead libraries (see above). Using purified secretory IgMs, derived from two murine B-lymphoma cell lines, as probes to screen a series of random peptide libraries (all L-amino acids or all D-amino acids), we have identified a number of peptides with different motifs that bind specifically to the corresponding lymphoma cell lines.[38] These peptides, when presented to the intact cells in a tetrameric[38] or octameric[34] form, can trigger signal transduction, as revealed by Western blot probed with an anti-phosphotyrosine antibody. Furthermore, these peptides can not only bind specifically to the lymphoma cell surface, they can also enter the cytoplasm and then the nucleus.[34]

If purified cell surface receptors are not available or if the receptor is unknown or unstable upon solublization with detergent, one may use intact cells as probes to screen the bead libraries. In these experiments, intact cells are mixed with the bead library and incubated for a period, and the beads coated with a layer of cells are then isolated for microsequencing.[39−41] A subtractive technique is used, which identifies beads that are false positives, after the positive beads from the first screen with the cell of interest are recycled and checked for nonspecific binding to unrelated cell lines. The negative beads from the second screening, which were positive in the first screening, are then collected and again tested for rebinding to the original cell line. Positive beads from this last step are true positive and specific beads. In other experiments, we have incorporated blocking antibodies against known receptors (e.g., anti-integrin antibody) in one of the screening steps so that we can determine which positive beads in the initial step indeed bind to the integrin receptor on the cell surface.[39] As an alternative method, we label one cell line with calcein AM (fluorescent green) and keep another unrelated cell line unlabeled.[34] The cell mixture is then incubated with the bead library and inspected under a fluorescent microscope with low background light. Beads that bind to both color and colorless cells are nonspecific. Those beads that bind to only one of the two cell types are specific. Using the above methods, we have identified cell surface ligands that bind to prostate cancer cell lines,[39] non-small cell lung cancer cell lines,[40] and Jurkat lymphoma cell lines.[41] We believe that many of these ligands bind specifically to the integrins of these cancer cells and that many of these ligands might be cancer specific. Work is currently underway in our laboratory to develop these ligands into therapeutic agents and to characterize their effects on cell signaling.

Identification of Peptidomimetic and Small Molecules that Interact with Intracellular Signaling Proteins

Our approach to functional proteomics is to develop encoded OBOC peptidomimetic and small-molecule libraries to probe whole cell extracts. When we mix one protein with 1 million compound beads, we are examining

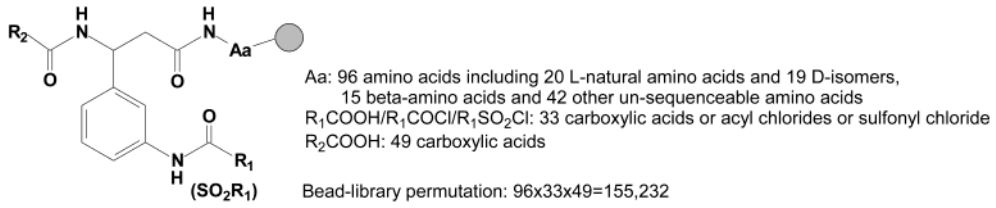

FIGURE 3. Chemical structure of β-amino acid-based peptidomimetic bead library.

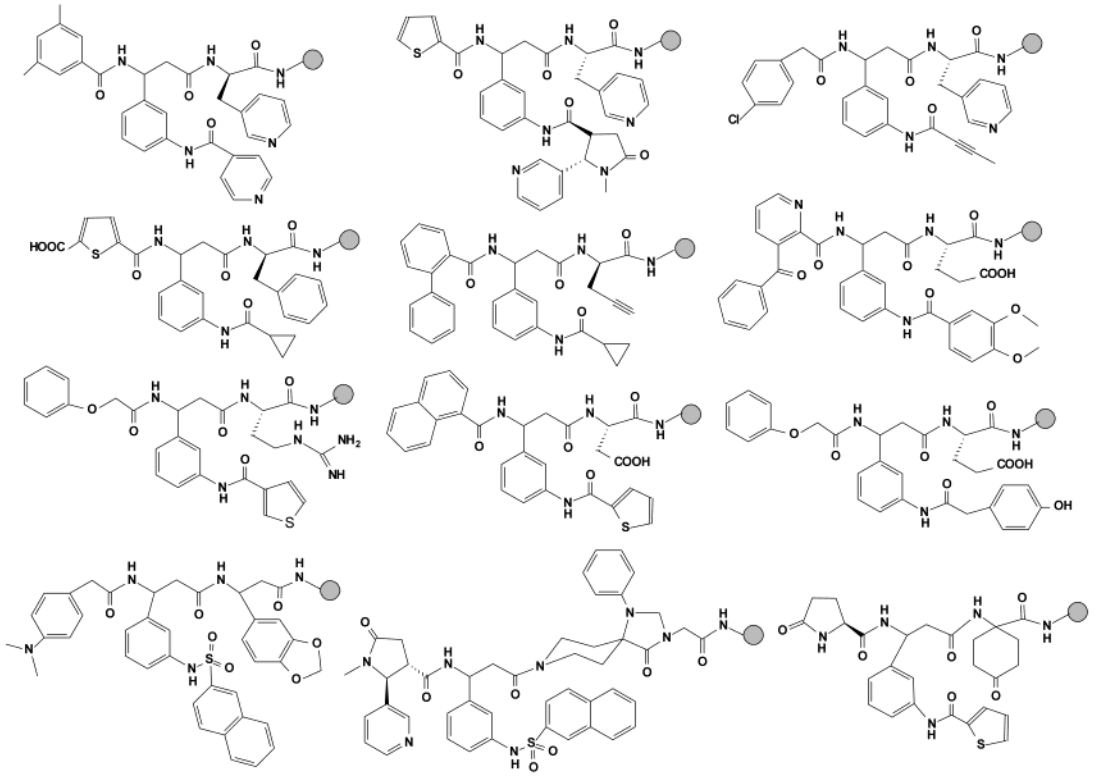

FIGURE 4. Chemical structure of ligands bind to Ramos cell extract (each ligand is attached to a bead via an amide bond and a long polyethylene−glycol linker).

1 million interactions simultaneously. When we mix the whole cell extract with 1 million compound beads, we are essentially studying multibillion interactions concurrently. Figure 2 summarizes our experimental approach. We first prepare cell extract, biotinylate it, and then incubate it with an encoded peptidomimetic or small-molecule bead library. After thorough washing, the library is treated with streptavidin−alkaline phosphatase complex, and BCIP is added for color development. The colored positive beads are then isolated for decoding. Once the structure of the compound is identified, they will be resynthesized in a solution form, and their biological and biochemical effects on intact cell will be evaluated. Additionally, these ligands will be resynthesized on Sepharose beads via a hydrophilic linker and these beads will be used to capture target protein(s) or protein complex(es) for subsequent identification. Those compounds that elicit a biological effect, such as morphological changes, apoptosis, or cell cycle perturbations, will be further characterized for effects on cell signaling. Once several hundreds of ligands are identified, they will be resynthesized and printed on a slide to form sets of microarrays. These microarray sets can then be used for functional profiling of cell extracts derived from normal tissue, cancer cell lines, or cancer biopsy specimens (see below) and then compared to the appropriate normal tissue cellular extract. This will allow for identification of cancer cell-specific ligands.

We have used this approach to analyze cell extracts derived from Ramos cell line, a human B-cell lymphoma cell line. The chemical structure of the peptidomimetic library used in our study is shown in Figure 3. Forty-five ligands have been identified, and some of their chemical structures are shown in Figure 4. A preliminary study of resynthesized ligands shows that some of these ligands have cytotoxic effects on Ramos cells. Work is currently underway in our laboratory to further characterize some of these ligands and to identify and characterize additional ligands for Ramos cell extract.

Chemical Microarray as a Tool to Profile Protein Kinase Substrates and Ligands for Intracellular Proteins and Cell Surface Receptors

As indicated above, we have developed methods to print peptides or small molecules on glass[19] or plastic slides[34]

in a microarray format. These microarrays can then be used to efficiently profile target molecules derived from various sources. Essentially all the different assays that we have developed for the OBOC libraries can also be used to analyze the microarrays. For example, we have printed peptide substrates for protein kinases on glass slides in a microarray format.[19] The protein kinase with [γ-^{33}P]ATP is then applied to the surface of the microarray. After incubating for 1 h, the slide is thoroughly washed and exposed to an X-ray film. The phosphorylation profile is depicted by the autoradiogram that can be scanned and quantitated. We have found that a long hydrophilic linker between the peptide and the glass surface is needed for effective phosphorylation of the microarray.

We have also reported the printing of cell binding peptide microarrays and used them to profile cell surface binding properties in a number of different cell types.[19,34] In these experiments, the polystyrene slide is first coated with neutravidin, and biotinylated peptides are then printed on the surface of the slide. Cell suspensions containing a lymphoma cell line, normal lymphocytes, or cells derived from cancer biopsy specimens or leukemia patient's peripheral blood are then applied to each of the replicate sets peptide microarray. After incubating for about 30 min, the slide is gently washed, and the bound cells are fixed with formaldehyde and stained with crystal violet. The slide is then scanned, and the relative amount of bound cells is quantitated.

In addition to quantitating cell adhesion to each microarray spot, we have also developed methods to evaluate the effect of cell adhesion on cell signaling.[19] In these experiments, after the bound cells are fixed with formaldehyde, the slide is treated with a nonionic detergent to permeate the cells. The microarray is then treated with fluorescent monoclonal antibodies that specifically detect signaling proteins, and finally the slide is inspected under a fluorescent microscope. To analyze more than one signaling protein, multicolor fluorescent-labeled antibodies can be used to probe the same microarray. If additional studies are needed, replicates of microarray can be used.

Perspectives

Combinatorial chemistry is an enabling technology that can be applied to many fields. The ability to generate many peptides, peptidomimetics, and small molecules and to use these techniques to rapidly probe signaling molecules along the pathway is a powerful approach to study cell signaling. Although this article only focuses on the use of OBOC combinatorial library and chemical microarray approaches in signal transduction research, other related combinatorial library methods are equally useful. The investigator should choose the technique on the basis of his/her expertise, the particular biological problem under study, and the availability of assays, reagents, and resources. The techniques described in this article enable one to use proteomics approaches to study signal transduction in a global fashion rapidly. As these methods continue to evolve, new complimentary techniques will be developed, assays are becoming highly miniaturized, thousands to tens of thousands of assays can be run in parallel, and only a minute quantity of reagents are needed for the analysis. In addition to increasing our understanding of nature, these techniques will also provide useful information in the development of drugs and diagnostic tools for many human diseases.

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