(54) Title: HUMAN BAD POLYPEPTIDES, ENCODING NUCLEIC ACIDS AND METHODS OF USE

(57) Abstract

The invention provides an isolated gene and an isolated nucleic acid sequence encoding human Bad and functional fragments thereof. Also provided is an isolated human Bad polypeptide and functional fragments thereof. Methods of identifying human Bad binding partners and methods of screening for compounds which interfere with the association of human Bad interacting polypeptides with human Bad are also provided. Finally, methods for decreasing or increasing the viability of a cell are provided as well.

HUMAN BAD POLYPEPTIDES, ENCODING NUCLEIC ACIDS AND METHODS OF USE

Throughout this application various publications are referenced within parentheses. The disclosures of
5   these publications in their entireties are hereby incorporated by reference in this application in order to more fully describe the state of the art to which this invention pertains.

BACKGROUND OF THE INVENTION

10   The present invention relates generally to apoptosis or, programed cell death, and more particularly, nucleic acids encoding human Bad polypeptides which can be used to modulate apoptosis for the therapeutic treatment of human diseases.

15   Apoptosis is a normal physiological process of cell death that plays a critical role in the regulation of tissue homeostasis by ensuring that the rate of new cell accumulation produced by cell division is offset by a commensurate rate of cell loss due to death. It has
20   now become clear that disturbances in apoptosis, also referred to as physiological cell death or programmed cell death, that prevent or delay normal cell turnover can be just as important to the pathogenesis of diseases as are known abnormalities in the regulation of
25   proliferation and the cell cycle. Like cell division, apoptosis is similarly regulated under normal circumstances through complex interaction of gene products that either promote or inhibit cell death.

The stimuli which regulate the function of
30   these apoptotic gene products include both extracellular

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and intracellular signals. Either the presence or the absence of a particular stimuli can be sufficient to evoke a positive or negative apoptotic signal. For example, physiological stimuli that prevent or inhibit

5   apoptosis include, for example, growth factors, extracellular matrix, CD40 ligand, viral gene products neutral amino acids, zinc, estrogen and androgens. In contrast, stimuli which promote apoptosis include growth factors such as tumor necrosis factor (TNF), Fas, and

10 transforming growth factor β (TGFβ), neurotransmitters, growth factor withdrawal, loss of extracellular matrix attachment, intracellular calcium and glucocorticoids, for example. Other stimuli, including those of environmental and pathogenetic origins, also exist which

15 can either induce or inhibit programmed cell death. Thus, apoptosis is mediated by diverse signals and complex interactions of cellular gene products which ultimately result in a cell death pathway that is required to regulate normal tissue homeostasis.

20   Several gene products which modulate the apoptotic process have now been identified. Although these products can in general be separated into two basic categories, gene products from each category can function to either inhibit or induce programmed cell death. One

25 family of gene products are those which are members of the Bcl-2 family of proteins. Bcl-2, is the best characterized member of this family and inhibits apoptosis when overexpressed in cells. Other members of this gene family include, for example, Bax, Bak, Bcl-x$_L$,

30 Bcl-x$_S$, and Bad. While some of these proteins can prevent apoptosis, others such as Bax, Bcl-x$_S$ and Bak function to augment apoptosis.

A second family of gene products which modulate
the apoptotic process is the family of proteases known as
aspartate-specific cysteine proteases (ASCPs). These
proteases are related genetically to the ced-3 gene

5   product which was initially shown to be required for
programmed cell death in the roundworm, *C. elegans*. The
ASCPs family of proteases includes, for example, human
ICE (interleukin-1-β converting enzyme), ICH-1$_L$, ICH-1$_S$,
CPP32, Mch2, Mch3, ICH-2 and ICE$_{rel}$·III. One common

10   feature of these gene products is that they are cysteine
proteases with specificity for substrate cleavage at Asp-
X bonds. Although these proteases induce cell death
when expressed in cells, several alternative structural
forms such as ICEδ, ICEε, ICH-1$_S$ and Mch2β are known which

15   actually function to inhibit apoptosis.

In addition to the Bcl-2 and ASCP gene families
which play a role in apoptosis in mammalian cells, it has
become increasingly apparent that other gene products
exist which are important in mammalian cell death and

20   which have yet to be identified. For example, in
addition to Ced-3, another *C. elegans* gene known as Ced-4
exists which is also required for programmed cell death
in *C. elegans*. However, mammalian homologues of this
protein remain elusive and have not yet been identified.

25   Further, it is ambiguous as to whether other genes exist
which belong to either of the above two apoptotic gene
families or what role they may play in the programmed
cell death pathway. Finally, it is also unclear what the
physiological control mechanisms are which regulate

30   programmed cell death or how the cell death pathways
interact with other physiological processes within the
organism.

The maintenance of tissue homeostasis is
required in a range of physiological processes such as

35   embryonic development and immune cell regulation as well

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as in normal cellular turnover. The dysfunction, or loss
of regulated apoptosis can lead to a variety of
pathological disease states. For example, the loss of
apoptosis can lead to the pathological accumulation of
5   self-reactive lymphocytes such as that occurring with
many autoimmune diseases. Inappropriate loss of
apoptosis can also lead to the accumulation of virally
infected cells and of hyperproliferative cells such as
neoplastic or tumor cells. Similarly, the inappropriate
10   activation of apoptosis can also contribute to a variety
of pathological disease states including, for example,
acquired immunodeficiency syndrome (AIDS),
neurodegenerative diseases and ischemic injury.

Treatments which are specifically designed to
15   modulate the apoptotic pathways in these and other
pathological conditions can change the natural
progression of many of these diseases. However, since
distinct differences are known to exist between human and
other species such as *C. elegans*, it is advantageous to
20   use human proteins for the production of
biopharmaceuticals as well as other molecules or
compounds which are produced to modify the function of a
regulator of programed cell death. The use of human
proteins as both biopharmaceuticals and to identify
25   compounds is also important in regard to the therapeutic
efficacy and safety of the treatment. For example, the
half-life of non-human proteins can be comprised in a
heterologous environment and the induction of a host
immune response against a non-human protein can be
30   potentially dangerous to the patient.

Thus, there exists a need to identify new
apoptotic genes and their human homologues and for
methods of modulating apoptotic process for the
therapeutic treatment of human diseases. The present

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invention satisfies this need and provides related
advantages as well.

SUMMARY OF THE INVENTION

The invention provides an isolated gene and an
5   isolated nucleic acid sequence encoding human Bad and
functional fragments thereof. Also provided is an
isolated human Bad polypeptide and functional fragments
thereof. Methods of identifying human Bad binding
partners and methods of screening for compounds which
10 interfere with the association of human Bad interacting
polypeptides with human Bad are also provided. Finally,
methods for decreasing or increasing the viability of a
cell are provided as well.

BRIEF DESCRIPTION OF THE DRAWINGS

15   Figure 1 shows the nucleotide and predicted
amino acid sequence of human Bad (SEQ ID NOS:1 and 2,
respectively).

Figure 2 shows a comparison of the amino acid
sequence between human Bad (SEQ ID NO: 2; clone PBM1) and
20 mouse Bad (SEQ ID NO: 3; PmBad). The boxed regions
emphasize amino acid identities and differences.

Figure 3 shows binding interactions between
human Bad and Bcl-2 and between human Bad and Bcl-X$_L$ in a
yeast two-hybrid assay.

25   Figure 4 shows the binding interactions between
human Bad and Bcl-2 and between human Bad and Bcl-X$_L$ in an
ELIZA assay.

DETAILED DESCRIPTION OF THE INVENTION

This invention is directed to the human Bcl-x_L/Bcl-2 associated death (Bad) promoting polypeptide and encoding nucleic acids. This protein is a member of the
5   Bcl-2 family of cell death regulatory proteins in that it shows sequence homology with this family of proteins and is able to bind Bcl-x_L and to Bcl-2. The binding of human Bad to Bcl-x_L results in the induction of programmed cell death. The binding properties of human Bad to Bcl-x_L have
10   been found to be different from the properties of Bax and Bak, two other death-enhancing Bcl-2 family proteins. For example, amino-terminal deletions in Bax which create a fragment leaving the BH-1 and BH-2 domains intact completely abolish the ability of this fragment to
15   interact with Bcl-X_L. In contrast, equivalent truncations of Bad leave the ability of this fragment to bind Bcl-x_L intact. This difference in interaction can have therapeutic advantages because compounds can be used which selectively target interactions of Bad and suppress
20   programed cell death without interfering with other cell death regulatory proteins.

In one embodiment, the invention is directed to nucleic acids encoding human Bad. The nucleic acids are used to produce recombinant Bad polypeptides which can be
25   used in screening assays to identify compounds that interfere with Bad function. These screening assays yield compounds that antagonize the function of Bad and thus will prevent apoptosis. Such pharmaceutical compounds are useful for the treatment or prevention of
30   diseases which are characterized by apoptotic cell death. Alternatively, assays can be configured to yield compounds that exhibit substantially the same function as, or "mimic" the function of Bad and thus enhance

apoptosis. These pharmaceutical compounds are useful for
the treatment or prevention of diseases which are
characterized by the loss of apoptotic cell death. Human
Bad polypeptides can also be used to identify novel

5   interacting polypeptides and to identify the binding
domains within these proteins as well as within known
binding polypeptides such as Bcl-X_L. Similarly, these
novel binding polypeptides and binding domains can be
used to inhibit or enhance apoptosis or to identify

10   compounds which modulate their apoptotic function.

As used herein, the term "substantially" when
referring to the human Bad nucleotide or amino acid
sequence is intended to refer to the degree to which two
sequences of between about 15-30 or more nucleotides in

15   length, are identical or similar so as to be considered
by those skilled in the art to be functionally
equivalent. For example, the human Bad nucleic acid of
the invention has a nucleotide sequence substantially the
same as that shown in Figure 1 and as SEQ ID NO:1. Thus,

20   if a second sequence is substantially the same as that
shown as SEQ ID NO:1 so as to selectively hybridize, then
it is considered functionally equivalent by those skilled
in the art. Methods for sequence comparisons and
determinations of similarity are well known and routine

25   within the art.

Functionally equivalent nucleic acid sequences
include, for example, sequences that are related, but
different and encode the same human Bad polypeptide due
to the degeneracy of the genetic code as well as

30   sequences that are related, but different and encode a
different human Bad polypeptide that exhibits similar
functional activity. In both cases, the nucleic acids
encode functionally equivalent gene products. Functional

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fragments of human Bad encoding nucleic acids such as
oligonucleotides, polyoligonucleotides, primers and
modified forms thereof are also considered to be within
the definition of the term and the invention as claimed.

5 Modified forms of functionally equivalent nucleic acid
sequences include, for example, chemical modifications or
derivatives such as those used in antisense therapy.
Functional equivalency is also relevant to human Bad
nucleic acids which do not encode gene products, for

10 example, but instead are functional elements in and of
themselves. Specific examples of such functional nucleic
acids include, for example, promoters, enhances and other
gene expression regulatory elements.

Human Bad polypeptides of the invention have an

15 amino acid sequence substantially similar to that shown
in Figures 1 and in SEQ ID NO:2. Functionally equivalent
human Bad amino acid sequences similarly includes, for
example, related, but different sequences so long as the
different polypeptide exhibits at least one functional

20 activity of human Bad. Specific examples of Bad
polypeptide functional activities include functions such
as the ability to bind or otherwise interact with Bad
interacting polypeptides and/or Bad binding partners and
the ability to promote cell death by heterdimerization

25 with, for example, Bcl-x$_L$ and Bcl-2. Related, but
different polypeptides also include, for example,
substitutions of conserved and/or non-essential amino
acids within the Bad polypeptide sequence. Fragments and
functional domains of human Bad are similarly included

30 within the definition of the term and the claimed
invention.

Therefore, it is understood that limited
modifications may be made without destroying the

biological function of the human Bad polypeptide and that

only a portion of the entire primary structure may be

required in order to effect activity. For example, minor

modifications of the human Bad amino acid sequence (SEQ

5   ID NO:2) which does not destroy its activity also fall

within the definition of human Bad and within the

definition of the polypeptide claimed as such. Also, for

example, genetically engineered fragments of human Bad

either alone or fused to heterologous proteins such as

10   fusion proteins that retain measurable enzymatic or other

biological activity fall within the definition of the

polypeptides as claimed.

     It is understood that minor modifications of

primary amino acid sequence may result in polypeptides

15   which have substantially equivalent or enhanced function

as compared to the sequences set forth in Figure 1 (SEQ

ID NO:2). These modifications may be deliberate, as

through site-directed mutagenesis, or may be accidental

such as through mutation in hosts which are human Bad

20   producers. All of these modifications are included as

long as human Bad biological function is retained.

Further, various molecules can be attached to human Bad,

including for example, other proteins, carbohydrates,

lipids, or chemical moieties. Such modifications are

25   included within the definition of human Bad polypeptides.

     As used herein, the term "Bad interacting

polypeptide" is intended to mean a peptide, polypeptide

or protein which interacts directly or indirectly with

the human Bad polypeptide. Such polypeptides include,

30   for example, Bcl-x$_L$ and Bcl-2. When associated with human

Bad, these interacting polypeptides form a heterodimer

with the human Bad polypeptide. Direct interaction can

include the direct binding of human Bad polypeptide with

the interacting polypeptide. Binding can occur, for
example, through polypeptide-polypeptide binding
interaction or through binding involving post-
translational modifications. Examples of post-
5 translational modifications can include phosphorylation,
glycosylation, acylation and lipidation. Indirect
interaction between Bad and Bad interacting polypeptides
can include, for example, the interaction of human Bad
with a docking protein such as an SH2 or SH3 containing
10 protein with functions to regulate the activity of a cell
death protein proximal in the human Bad pathway. Human
Bad interacting polypeptides can enhance Bad activity
such as by augmenting the interaction of Bad with Bcl-x$_L$
or Bcl-2 or with other cell death regulatory molecules.
15 Alternative, human Bad interacting polypeptides can
inhibit Bad activity by preventing the interaction of Bad
with, for example, Bcl-x$_L$, Bcl-2 or other cell death
regulatory molecules. Similarly, such interactions can
occur directly or indirectly.

20   As used herein, the term "human Bad binding
partner" is intended to mean polypeptides,
non-polypeptide mimetics, organic molecules and other
compounds which bind to the human Bad polypeptide. As
with human Bad interacting polypeptide, human Bad binding
25 partners can either inhibit or enhance the function of
Bad through the binding of these partners to human Bad.

          The invention provides a gene encoding human
Bad or functional fragment thereof. The invention also
provides an isolated nucleic acid sequence encoding human
30 Bad or fragment thereof. The gene and nucleic acid
sequences encode substantially the sequence as shown in
SEQ ID NO:1. Fragments of the gene or nucleic acid
sequence are provided which comprise single or double

stranded nucleic acids having substantially the sequences
shown in SEQ ID NO:1.

     The human Bad nucleic acids of the present
invention were identified by searching a human database
5 of expressed sequence tags (ESTs) to identify potential
new sequence fragments which may have homology to the
mouse Bad gene and other Bcl-2 family cell death
regulators. This approach was chosen because the
standard approach of using the mouse sequence to probe
10 human cDNA libraries had failed in this case as described
in the Examples. Searches of the EST database identified
short ESTs containing partial nucleotide sequence
homology to the mouse Bad nucleotide sequence. These EST
sequences were then used to design probes for the
15 screening of cDNA libraries. Screening with the EST
derived probes resulted in the identification of a
putative human Bad nucleotide sequence which encoded a
protein with approximately 77% amino acid homology to
mouse Bad with the exception of an apparent truncation of
20 42 amino acids at the amino terminus. A comparison of
the amino acid sequence between human Bad (SEQ ID NO: 2)
and mouse Bad (SEQ ID NO: 3) is shown in Figure 2.
Unexpectedly, this apparent truncation has now been shown
to be the authentic amino terminus of human Bad.

25   In regard to the search for potential new
sequences having homology to mouse Bad and other Bcl-2
family members, there are numerous considerations which
must be taken into account when searching for homologies
from unknown sequences such as ESTs fragments. For
30 example, searching a genetic data base will yield
homologous sequence matches to essentially any query
nucleotide sequence. However, in order for the search to
result in a meaningful match, additional criteria and

permutations must be used to identify the authentic
homologue of interest from among the non-specific
homology matches. Mouse Bad shares the highest degree of
homology with other Bcl-2 related proteins in the region

5   believed to contain the binding site with Bcl-X_L and
Bcl-2. A given EST returned by the search may not
include one of these functionally important highly
homologous sites, but rather, may only include a region
within the polypeptide with cryptic homology. Confirming

10 an EST as an actual Bad or BH1 and BH2 encoding
polypeptide involves translation of all positive EST hits
in three different reading frames and subsequent
identification of conservative sequence homologies to the
gene of interest. Then, using conventional cDNA cloning,

15 a full length cDNA of the putative novel gene can be
obtained and 1) analyzed for overall structural homology
to Bad and Bcl-2 family members, 2) recombinantly
expressed and analyzed for binding activity to other
Bcl-2 family proteins and 3) analyzed for the induction

20 or suppression of programmed cell death by heterologous
expression of the cDNA in appropriate cells.

Alternative methods than that described above
for isolating human Bad encoding nucleic acids can
similarly be employed. For example, using the teachings

25 described herein, those skilled in the art can routinely
isolate and manipulate human Bad nucleic acids using
methods well known in the art. All that is necessary is
the sequence of the human Bad encoding nucleic acids
(Figures 1 and SEQ ID NO:1) or its amino acid sequence

30 (Figures 1 and SEQ ID NO:2). Such methods include, for
example, screening a cDNA or genomic library by using
synthetic oligonucleotides, nucleic acid fragments or
primers as hybridization probes. Alternatively,
antibodies to the human Bad amino acid sequence or

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fragments thereof can be generated and used to screen an expression library to isolate human Bad encoding nucleic acids. Other binding reagents to human Bad polypeptides, such as Bcl-X$_L$ or fragments thereof can similarly be used

5   to isolate human Bad polypeptides having substantially the amino acid sequence show in Figure 1.

In addition, recombinant DNA methods currently used by those skilled in the art include the polymerase chain reaction (PCR) which, combined with the human Bad

10 nucleotide and amino acid sequences described herein, allows reproduction of human Bad encoding sequences. Desired sequences can be amplified exponentially starting from as little as a single gene copy by means of PCR. The PCR technology is the subject matter of United States

15 Patent Nos. 4,683,195, 4,800,159, 4,754,065, and 4,683,202 all of which are incorporated by reference herein.

The above described methods are known to those skilled in the art and are described, for example, in

20 Sambrook et al., <u>Molecular Cloning: A Laboratory Manual</u>, Cold Spring Harbor Laboratory, New York (1992) and the various references cited therein and in Ansubel et al., <u>Current Protocols in Molecular Biology</u>, John Wiley and Sons, Baltimore, MD (1989); and in Harlow et al.,

25 <u>Antibodies: A Laboratory Manual</u>, Cold Spring Harbor Laboratory, New York (1989). These references and the publications cited therein are hereby expressly incorporated herein by reference.

The invention provides an isolated human Bad

30 polypeptide comprising substantially the amino acid sequence as that shown in Figure 1 (SEQ ID NO:2). Human Bad functional fragments are also provided. A specific

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example of a human Bad functional fragment is the Bcl-X$_L$
binding domain.

Isolated human Bad polypeptides of the
invention can be obtained by a variety of methods known
5   within the art. For example, the isolated peptides can
be purified by biochemical methods including, for
example, affinity chromatography. Affinity matrices
which can be used for human Bad isolation can be
anti-human Bad monoclonal or polyclonal antibodies
10   prepared against the amino acid sequence shown in Figure
1 (SEQ ID NO:2), or fragments thereof such as synthetic
peptides, recombinant fragments or the like.
Alternatively, cognate binding domains or polypeptides as
well as other compounds known in the art which
15   specifically bind to human Bad can similarly be used as
affinity matrices to isolate substantially pure human Bad
polypeptides of the invention.

Human Bad polypeptides can also be produced by
recombinant methods known to those skilled in the art.
20   Recombinant human Bad polypeptides include, for example,
an amino acid sequence substantially the same as that
shown in Figures 1 (SEQ ID NO:2) as well as fusion
proteins and fragments thereof. The human Bad encoding
nucleic acids can be cloned into the appropriate vectors
25   for propagation, manipulation and expression. Such
vectors are known or can be constructed by those skilled
in the art and should contain all expression elements
necessary for the transcription, translation, regulation,
and if desired, sorting of the human Bad polypeptides.
30   The vectors can also be for use in either prokaryotic or
eukaryotic host systems so long as the expression and
regulatory elements are of compatible origin. One of
ordinary skill in the art will know which host systems

are compatible with a particular vector. The recombinant
polypeptides produced can be isolated by, for example,
the methods described above.

5   Apoptosis plays a significant role in numerous
pathological conditions in that programmed cell death is
either inhibited, resulting in increased cell survival,
or enhanced which results in the loss of cell viability.
Examples of pathological conditions in which increased
cell survival contributes to the disease process include
10   cancers in general, and more particularly cancers such as
lymphomas and hormone dependent tumors. Such hormone
dependent tumors include, for example, breast, prostate
and ovarian cancer. Inhibition of programed cell death
of immune cells plays a role in autoimmune diseases such
15   as systemic lupus erythematosus and immune-mediated
glomerulonephritis. Viruses such as herpesvirus,
poxvirus and adenovirus carry anti-apoptotic genes to
prolong production of viral particles by the host cell by
preventing the host cell from committing suicide.

20   In contrast, apoptotic diseases where enhanced
programed cell death is part of the disease process
generally include, for example, neurodegenerative
disorders such as Alzheimer's disease, Parkinson's
disease, Amyotrophic lateral sclerosis, Retinitis
25   pigmentosa, and Cerebellar degeneration. Other diseases
associated with increased apoptosis include, for example,
myelodysplastic syndromes such as aplastic anemia and
ischemic injury including myocardial infarction, stroke
and reperfusion injury.

30   The human Bad encoding nucleic acids and
polypeptides of the invention can be used to diagnose,
treat or reduce the severity of cell death mediated

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diseases such as those described above as well as other
diseases mediated by either increased or decreased
programmed cell death. Additionally, the human Bad
encoding nucleic acids and polypeptides of the invention
5   can be used to screen for pharmaceutical compounds and
macromolecules which inhibit or promote human Bad
mediated programmed cell death.

For example, the human Bad encoding nucleic
acids, polypeptides and functional fragments thereof can
10   be used to diagnose, or to generate reagents to diagnose
diseases mediated or characterized by programed cell
death. Diagnosis can be by, for example, nucleic acid
probe hybridization with human Bad containing nucleotide
sequences or by antibody or ligand mediated detection
15   with human Bad binding agents. Such methods are routine
to those skilled in the art. Detection can be performed
ex vivo, for example, by removing a cell or tissue sample
from an individual exhibiting or suspected of exhibiting
a cell death mediated disease. Correlation of increased
20   human Bad expression or activity is indicative of
diseases characterized by enhanced programmed cell death
whereas correlation of decreased human Bad expression or
activity is indicative of diseases characterized by the
suppression of programmed cell death.

25   The above human Bad polypeptides can also be
formulated into pharmaceutical compositions known within
the art for the treatment of cell death mediated diseases
characterized by increased cell survival and
proliferation. Functional fragments, including peptides
30   and peptide mimetics corresponding to the Bcl-X_L binding
domain of human Bad can similarly be formulated for the
treatment of such diseases associated with increased cell
survival and proliferation. Administration of human Bad

polypeptides and functional fragments thereof will induce apoptosis in treated cells and eliminate those cells characterized by increased cell survival or proliferation. Administration of non-human Bad

5 polypeptides, molecules or compounds that induce the activation, regulation or expression of human Bad can similarly be used for the treatment of diseases characterized by increased cell survival and proliferation. Specific examples of such polypeptides,

10 molecules and compounds include Bad interacting polypeptides, binding partners and non-peptide compound equivalents.

To be effective, the human Bad polypeptides must be introduced into the cells characterized by

15 increased cell survival. Introduction can be accomplished by a variety of means known within the art including, for example, lipid vesicles and receptor mediated endocytosis. Targeting to the appropriate cell type can similarly be accomplished through conjugation to

20 specific receptor ligands, specific target cell antibodies and the like.

The human Bad polypeptides are administered by conventional methods, in dosages which are sufficient to induce apoptosis in the cells characterized by increased

25 cell survival or proliferation. Such dosages are known or can be easily determined by those skilled in the art. Administration can be accomplished by, for example, intravenous, intraperitoneal or subcutaneous injection. Administration can be performed in a variety of different

30 regimens which include single high dose administration or repeated small dose administration or a combination of both. The dosing will depend on the cell type, progression of the disease and overall health of the

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individual and will be known or can be determined by
those skilled in the art.

In contrast to the induction of human Bad
mediated apoptosis for the treatment of pathological
5   conditions characterized by increased cell survival or
proliferation, inhibitors of human Bad can be used to
treat diseases characterized by increased programmed cell
death. Such inhibitors can be, for example, binding
domain inhibitors, including small non-peptide, organic
10 molecule inhibitors. Such inhibitors are formulated in a
medium which allows introduction into the desired cell
type. Alternatively, such inhibitors can be attached to
targeting ligands for introduction by cell mediated
endocytosis and other receptor mediated events.

15   Other inhibitors of human Bad include, for
example, small molecules and organic compounds which bind
and inactivate human Bad by a competitive or non-
competitive type mechanism. Molecules, polypeptides or
compounds which indirectly inhibit the human Bad pathway
20 can also be used as inhibitors of human Bad. Indirect
inhibition can be accomplished by, for example,
inhibition of Bad interacting polypeptides with Bad.
Such inhibition includes, for example, the inhibition of
binding events as well as post-translational
25 modifications which function in promoting human Bad
mediated cell death. Human Bad inhibitors can be
identified by screening for molecules which demonstrate
specific or beneficial human Bad inhibitory activity.
Such methods are described further below and can be
30 practiced by those skilled in the art given the human Bad
nucleotide and amino acid sequences described herein.

Treatment or reduction of the severity of cell death mediated diseases can also be accomplished by, for example, introducing expressible nucleic acids encoding human Bad polypeptides or functional fragments thereof

5   into cells characterized by such diseases. For example, elevated synthesis rates of human Bad can be achieved by, for example, using recombinant expression vectors and gene transfer technology. Similarly, treatment or reduction of the severity of cell death mediated diseases

10   can also be accomplished by introducing and expressing antisense human Bad nucleic acids so as to inhibit the synthesis rates of human Bad. Such methods are well known within the art and include, for example, the introduction of a compatible expression vector into

15   population of cells characterized by either increased or decreased programmed cell death. Vectors can include, for example, both DNA and RNA viral vectors. Adenovirus is a specific example of a DNA viral vector.

Other methods of antisense inhibition known in

20   the art include, for example, the use of synthetic oligonucleotides which are permeable to the cell membrane. Such oligonucleotides can include methylphosphonate and thiophosphonate chemical moieties for facilitating membrane permeability. The

25   oligonucleotide can be employed for antisense inhibition of RNA processing, transport or translation events, or alternatively, oligonucleotides which form triple helices can be used for the inhibition of transcription. Such methods are well known in the art.

30   The vector to be used in the methods of the invention will depend on desired cell type to be targeted. For example, if neurodegenerative diseases are to be treated by decreasing the human Bad activity of

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affected neuronal cells then a vector specific for cells
of the neuronal cell lineage should be used. Likewise,
if diseases or pathological conditions of the
hematopoietic system are to be treated, than a viral
5   vector that is specific for blood cells and their
precursors, preferably for the specific type of
hematopoietic cell, should be used. Moreover, such
vectors can additionally be modified with specific
receptors or ligands and the like to modify or alter
10   target specificity through receptor mediated events.
These modification procedures can be performed by, for
example, recombinant DNA techniques or synthetic
chemistry procedures. The specific type of vector will
depend upon the intended application. The actual vectors
15   are also known and readily available within the art or
can be constructed by one skilled in the art using
methodology well known to those skilled in the art.

Vectors containing human Bad nucleic acids or
inhibitors of human Bad such as antisense nucleic acids
20   can be administered in several ways to obtain expression
and therefore either increase or decrease the activity of
human Bad in the cells affected by the disease or
pathological condition. If viral vectors are used, for
example, the procedure can take advantage of their target
25   specificity and consequently, do not have to be
administered locally at the diseased site. However,
local administration can provide a quicker and more
effective treatment. Administration can also be
performed by, for example, intravenous or subcutaneous
30   injection into the subject. Injection of the viral
vectors into the spinal fluid can also be used as a mode
of administration, especially in the case of
neurodegenerative diseases.

Transfection vehicles such as liposomes can be used to introduce the non-viral vectors into recipient cells within and inoculated area. Such transfection vehicles are known by one skilled within the art.

5   Alternatively, non-targeting vectors can be administered directly into a tissue of any individual. Such methods are known within the art and are described by, for example, Wolff et al. (*Science* 247:1465-1468 (1990)).

Additional features can be added to the vectors

10   to ensure safety and/or enhance therapeutic efficacy. Such features include, for example, markers that can be used to negatively select against cells infected with the recombinant virus. An example of such a negative selection marker is the TK gene described above that

15   confers sensitivity to the antibiotic gancyclovir. Negative selection is therefore a means by which infection can be controlled because it provides inducible suicide through the addition of antibiotic. Such protection ensures that if, for example, mutations arise

20   that produce mutant forms of human Bad, dysfunction of apoptosis will not occur.

As described previously, the human Bad encoding nucleic acids and human Bad polypeptides of the invention can be used to screen for compounds which inhibit or

25   enhance the activity of human Bad. Such compounds can act directly or indirectly on human Bad, human Bad binding partners or human Bad interacting polypeptides. Such screening methods are known to those skilled in the art and can be performed by either *in vitro* or *in vivo*

30   procedures. For example, described in Example II is a specific *in vivo* assay for human Bad binding activity. Also described therein is the identification of a human Bad binding domain which can be used as an inhibitory

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compound for human Bad binding activity. Moreover, this
binding domain can also be used to model alternative
inhibitors of human Bad and human Bad binding partners.
Methods other than that described in Example II can also
5   be used to screen and identify compounds which inhibit
human Bad. Specific examples include the *in vitro*
screening of small molecule libraries such as those
produced by combinatorial chemistry. Phage display
peptide libraries can similarly be screened for
10 inhibitory polypeptides. Such libraries offer the
advantages of producing greater than 10^8 peptide sequences
which can be screened in a single round of panning.
These methods as well as others are known within the art
and can be utilized to identify compounds which inhibit
15 or enhance human Bad binding activity.

It is understood that modifications which do
not substantially affect the activity of the various
embodiments of this invention are also included within
the definition of the invention provided herein.
20 Accordingly, the following examples are intended to
illustrate but not limit the present invention.

EXAMPLE I

Cloning And Characterization of Human Bad

This Example shows the cloning and sequence
25 analysis of human Bad. The results described herein
indicate that human Bad is a unique member of the BH1 and
BH2 containing cell death regulatory polypeptides.

For the isolation of the human bad cDNA, an EST
database was searched using the mouse protein sequence as
30 a search criteria. However, prior to the use of the
below described approach using expressed sequence tags

(EST), two human c-DNA libraries were initially screened
with a mouse bad probe under low stringency conditions.
No human bad clones were identified using this approach.
An EST derived probe subsequently led to the isolation of
5   human bad c-DNA clones from the same libraries.

Subsequent to the above conventional approach,
the EST database (NCBI) was searched with a 204 amino
acid mouse protein sequence using the BLAST network
service.   Initial search attempts resulted in partial
10   sequences showing limited homology to the search query.
Briefly, an EST sequence was identified that had 25 out
of 32 identical amino acids in the region corresponding
to mouse residues 100-131 and 7 out of 7 identical amino
acids in a region corresponding to mouse residues
15   147-153. Overall, this EST sequence was 364 nucleotides
in length and exhibited an overall sequence homology to
the mouse sequence of 72% at the nucleotide level.
Differences in nucleotide sequence between the actual EST
and the corresponding mouse sequence were apparent and
20   likely resulted from no less than 37 sequencing errors
(additions, deletions and ambiguities) causing amino acid
frame shifts. As a result, only 32 out of a potential
109 amino acids (29%) actually aligned with the query
sequence. Nevertheless, this partial EST sequence
25   homology tentatively identifies a partial cDNA encoding
human Bad (EST #171560, GenBank #H18135) and was used to
screen human cDNA libraries so as to identify,
characterize and confirm the existence of the human Bad
cDNA and polypeptide.

30   Human cDNA libraries were screened using a 200
bp fragment amplified from the above putative human Bad
EST. The 200 bp fragment was labeled with ^{32}P-dCTP and
then used to screen libraries derived from human

24

substantia nigra, bone marrow and placenta. Screening
methods were used were those known in the art and which
are essentially described in Sambrook et al., _Molecular_
Cloning: A Laboratory Manual, Cold Spring Harbor
5   Laboratory, New York (1992). Briefly, 10^6 plaque forming
units were screened per library using a commercially
available hybridization buffer, Quick Hybe (Stratagene)
containing the above described putative human Bad EST
probe. To identify positive plaques, duplicate filters
10   were washed three times in 1X SSC and exposed to X-OMAT
film (Kodak).

        Screening of the each library resulted in the
identification of putative cDNAs encoding human Bad.
Surprisingly, none of these cDNAs appeared to be full
15   length when compared to the corresponding mouse Bad
sequence. For example, two partial cDNA's were isolated
from substantia nigra and encoded 95 amino acids of the
3' end of Bad. Similarly, seven partial cDNAs were
isolated from a placental cDNA library and three from a
20   bone marrow library. Again, these cDNAs were truncated
in that they encoded sequences substantially shorter than
the mouse Bad sequence. A sequence comparison of each of
these cDNAs revealed that all shared identical nucleotide
sequence in the region identified as the open reading
25   frame. However, when compared to the mouse Bad
nucleotide and predicted amino acid sequence all of these
isolated clones appeared to be truncated at their 5'
ends.

        Further sequence analysis of each of these
30   clones revealed that the longest clone, termed BM1,
exhibited a truncation of approximately 42 amino acids
compared to the initiation methionine of the mouse
sequence. Nevertheless, these apparently partial human
Bad cDNA clones exhibited a predicted open reading frame
35   that initiated with an amino terminal methionine. A

25

further comparison of the nucleotide and encoding amino
sequence revealed a 84% nucleotide identity and a 77%
amino acid identity when compared to the mouse sequence.
Thus, the similarity in sequence identity together with
5   the apparent amino terminal truncation lead to the
initial conclusion that the longest of the human Bad cDNA
clones was probably less than full length. Surprisingly
however, after further attempts to isolate a longer clone
and after numerous experimentations that independently
10 assessed the apparent authenticity of the methionine as a
translation initiator present in human clones, it was
concluded that the BM1 clone (SEQ ID NO:1) and shown in
Figure 1 as human Bad was the full length human sequence.
The predicted amino acid sequence of human Bad is also
15 shown in Figure 1 and as SEQ ID NO:2. Figure 2 shows the
amino acid alignments of human and mouse Bad. The
experimentation yielding these conclusions are described
further below.

To verify that cDNA BM1 encodes full length
20 human Bad, the 5' sequence of a number of different cDNAs
were analyzed. The results of that analysis revealed
that seven different human placenta clones and three
different human bone marrow clones terminated less that
54 nucleotides upstream from the above described proposed
25 start methionine. In addition, two subsequently entered
EST sequences of 317 and 300 nucleotides in length and
having some similarlity to the above described human Bad
cDNA were also found to terminate in this region. In all
cases, the sequence around the start methionine satisfied
30 Kozac criteria (A at-3 position). Also isolated were two
clones from placenta (PL14 and PL21) which differed from
other isolates in sequence just 5' to above described
start methionine. In contrast, the sequence in this 5'
region contains a consensus site for an RNA splicing
35 junction and is similar to the mouse Bad sequence. Taken

together, the above results indicate that there can be
alternatively spliced forms of human Bad.

EXAMPLE II

Human Bad Binding Partners

5   This Example shows that Human Bad can bind to
Bcl-X$_L$.

           To assess whether Bcl-2 family members bind to
or otherwise interact with human Bad and to map their
binding domains, a yeast two-hybrid system was employed
10 using the above described clones. Briefly, the full
length human Bad sequence was subcloned into the yeast
2-hybrid vector pEGE202, or "bait-vector" to express Bad
as a fusion protein with the DNA-binding protein LexA
(Ruden et al., Nature (1991) 350:426-430). Human Bcl-2,
15 Bcl-X, Bax, Bak and Bad were similarly cloned into the
yeast 2-hybrid vector pJG4-5, or "prey-vector" to
expressed these Bcl-2 family members as fusion proteins
with the transcriptional activator B42 (Gyuris et al.,
Cell (1993) 75:791-803). Briefly, the full length human
20 Bad sequence was amplified by PCR and subcloned into the
bait-vector. For the PCR reaction we used the following
primers: 5'ATC AGT GAA TTC ACT ATG TTC CAG ATC CCA GAC3'
(SEQ ID NO: 4) and 5'ATC GAT CTC GAG TCA CTG GGA GGG GGC
GGA GCT3' (SEQ ID NO: 5). The resultant PCR fragment was
25 digested with EcoRI and Xho I and then subcloned into the
bait vector. The human Bad sequence was also PCR
amplified and subcloned into the prey-vector using the
same primers and subcloning strategy as described above.
Human Bak was PCR amplified and subcloned as an
30 EcoRI/XhoI fragment into vector pJG4-5 using the
following primers: 5'ATC AGT GAA TTC ACT ATG GCT TCG GGG
CAA GGC CC-3' (SEQ ID NO: 6) and 5' ATC GAT CTC GAG TCA
GTT CAG GAT GGG ACC ATT GC-3 (SEQ ID NO: 7). Human Bax
was PCR amplified and subcloned as an EcoRI/XhoI fragment

27

into vector pJG4-5 using the following primers: 5' ATC
AGT GAA TTC ACT ATG GAC GGG TCC GGG GAG-3' (SEQ ID NO: 8)
and 5'-TAC AGT CTC GAG TCA GGT CAC GGT CTG CCA CGT GGG-3
(SEQ ID NO: 9).   Human Bcl-2, Bcl-X-L and Bcl-X-S in the

5   yeast 2-hybrid vector pJG4-5 were available and used as
described previously by Sato et al. (*Proc. Natl. Acad.
Sci.* 91:9238-9242 (1994)).

To determine if human Bad binds to any of the
above Bcl-2 family members, the Bad-bait plasmid was

10   cotransformed with every prey plasmid and the reporter
plasmid p18-34 into the yeast strain EGY191.   Cells were
induced for 6 hours with galactose to allow expression
from the prey plasmid.   Expression was assayed by lysing
a 100 ul aliquot of yeast cells (OD 600=0.6) in 96 well

15   plates in Z-buffer (60 mM Na_2HPO_4, 40 mM NaH$_2$PO4 x H_2O, 10
mM KCL, 1 mM $MgSO_4$ x 7 H$_2$0) and 1 u Lyticase (Boehringer
Mannhein) for 1 hour followed by addition of the
substrate ONPG into a final concentration of 2 mg/ml
(Sigma, St. Louis, MO).   The reaction was stopped with

20   0.4 M NaOH and the plate was read at an OD 420 nm.
Protein-protein interaction was measured as
beta-galactosidase activity using the following equation:
OD 420:(OD 600 x 0.1 ml x ONPG reaction time).

Results of these experiments showed that human

25   Bad interacts with Bcl-2 and Bcl-X_L.   However, no
interactions were observed between human Bad and Bax, Bak
or itself.   These results are presented in Figure 3.

EXAMPLE III

In Vitro **Screening and Identification of Compounds which**

30   **Modify the Binding Activity of Human Bad**

This Example shows the screening and
identification of compounds which inhibit human Bad

interacting polypeptides and human Bad associations using
recombinant human Bad polypeptides.

To screen for compounds which disrupt
associations between human Bad interacting polypeptides
and human Bad, an ELISA-type assay is employed. This
assay format is used here due to its reliability and
simplicity, however, other formats and binding assays can
be substituted in place of the ELISA. In particular, the
yeast-2-hybrid interaction between Bad and Bcl-X$_L$ or Bcl-2
shown in Example II can also be used as a compound
screening assay.

To generate fusion proteins for these screening
assays, Bad and Bcl-xL were cloned into pET-15b (Novagen)
and pGEX 4T1 (Pharmacia) vectors, respectively. The
pET15b-bad construct was made as follows. Full length
bad cDNA was amplified by PCR using cDNA BM1 as template
and primers containing NdeI (5') and XhoI (3')
restriction sites (5' primer: 5'-GGG AAT TCC ATA TGT TCC
AGA TCC CAG AG-3' (SEQ ID NO: 10)); 3' primer: 5'-TAC
AGT CTC GAG TCA CTG GGA GGG GGC GGA GCT-3' (SEQ ID
NO: 11)). The PCR product was then digested with
NdeI/XhoI and ligated into the NdeI and XhoI sites of
pET15b. The above primer sequences were chosen such that
the bad ORF sequence would be in frame with 5' vector
sequence encoding a six residue Histidine tag. The final
construct was termed pET-15 6H Bad.

The pGEX-Bcl-X$_L$ and pGEX Bax were constructed in
similar fashion using primers containing EcoRI sites on
both 5' and 3' ends (Bcl-X$_L$, 5' primer: 5'-AGT ATC GAA
TTC ATG TCT CAG AGC AAC CGG-3' (SEQ ID NO: 12), BcL-X$_L$
3' primer: 5'-ATT GAT GAA TTC GTT GAA GCG TTC CTG GCC
CTT-3' (SEQ ID NO: 13), Bax-5' primer: 5'-ATC AGT CTC
GAG ACT ATG GAC GGG TCC GGG GAG-3' (SEQ ID NO: 14),
Bax-3' primer: 5'-TAC GAT GAA TTC GGT CAC GGT CTG CCA

CGT GGG-3' (SEQ ID NO: 15). PCR products were then
digested with EcoRI and ligated into the EcoRI site of
pGEX 4T1. The above primer sequences were chosen such
that Bcl-X$_L$ and Bax ORF sequence would be in frame with 5'
5   vector sequence encoding a glutathione-S-transferase tag.

Proteins were purified according to methods
known in the art and to protocols provided by the vendor.
Briefly, pET 15-6Hbad was transformed into E. coli strain
BL21 plysS and pGE constructs into strain XL1-Blue for
10   protein production. Bacteria were grown in the presence
of 100 µg/ml carbenicillin (and 34 µg/ml chloramphenicol
in the case of BL21 plysS) until reaching an O.D of 1.
The cultures were induced with 1 mM IPTG and grown for an
additional 3 hours. Bacteria were harvested by
15   centrifugation, and protein isolated by sonication in 50
mM Tris, pH7 with 0.1% Triton X-100 containing protease
inhibitors PMSF, leupeptin (1 mg/ml) and benzamidine (1
mM). Proteins were then purified by affinity
chromatography using either nickel (for 6HBad) or
20   glutathione (for GST Bcl-X$_L$ and GST-Bax) as the affinity
ligand.

To perform the screening assays, multiwell
plates were coated overnight at 4°C with 50 µl/well of
the 6HBad fusion protein (4µg/ml) in phosphate buffered
25   saline (PBS). The plates were then washed twice with
phosphate-buffered saline containing 0.02% Tween (PBST)
and blocked with PBS containing 1% BSA (150 µl/well) for
2 hours at room temperature. Prior to addition of
compounds to be tested, the plates were washed twice with
30   PBST. Test compounds were added to the wells to a final
concentration of about 20µM (50 µl/well) in PBST + 0.5%
BSA. The plates were then incubated for 1 hour at room
temperature. GST-Bcl-x$_L$ fusion protein (liquid phase) at
about 1.5 µg/ml in PBST + 0.5% BSA was then added to the
35   plates (50µl/well) and incubated for 2 hours at room

30

temperature.   As a positive control, GST-Bcl-x$_L$ was added
in the absence of the test compound.   For a negative
control, assays were performed on plates not coated with
the 6HBad fusion protein.

5   An ELISA assay was then performed by first
washing the plates five times with PBST and then adding
an anti-GST monoclonal antibody (1:5000) in PBST + 0.5%
BSA to the well (50μl/well).   This antibody will detect
the GST-Bcl-x$_L$ fusion protein that remains bound to the
10   human Bad polypeptide on the plates.   The antibody was
allowed to incubate for 1 hour at room temperature.
Plates were washed five times with PBST and then
incubated with goat-anti-mouse-alkaline phosphatase
conjugated secondary antibody (1:1000) in PBST + 0.5% BSA
15   (50 μl/well).   The secondary antibody is allowed to
incubate for 1 hour at room temperature.   The plates were
washed five times with PBST and then incubated for 20
minutes with pNPP substrate (50μl/well).   Development of
the plates was stopped by addition of 0.4M NaOH
20   (50μl/well).   The plates were then read in a Molecular
Devices plate reader at 405 nm.   Compounds which inhibit
the association of human Bad interacting polypeptides
such as the GST-Bcl-x$_L$ fusion protein described above will
result in a lower signal compared to the positive
25   control.

Results of these experiments showed that human
6HBad interacts with GST-Bcl-X$_L$ and GST-Bcl-2, but not
with GST-Bax.   6HBad binds with highest affinity to
GST-Bcl-x$_L$.   These results are presented in Figure 4.

30   Although the invention has been described with
reference to the disclosed embodiments, those skilled in
the art will readily appreciate that the specific
experiments detailed are only illustrative of the

invention. It should be understood that various
modifications can be made without departing from the
spirit of the invention. Accordingly, the invention is
limited only by the following claims.

32

What is claimed is:

1.   An isolated gene encoding human Bad, or functional fragment thereof.

2.   The isolated gene of claim 1, comprising substantially the coding sequence in SEQ ID NO:1.

3.   The isolated gene of claim 1, wherein said functional fragment comprises single or double stranded nucleic acids of the sequence shown in SEQ ID NO:1.

4.   The isolated gene of claim 1, wherein said functional fragment comprises coding or non-coding strands of the sequence shown in SEQ ID NO:1.

5.   An isolated nucleic acid sequence encoding human Bad, comprising substantially the sequence shown in SEQ ID NO:1, or functional fragment thereof.

6.   The isolated nucleic acid sequence of claim 5, wherein said functional fragment comprises single or double stranded nucleic acids of the sequence shown in SEQ ID NO:1.

7.   The isolated nucleic acid sequence of claim 5, wherein said functional fragment comprises coding or non-coding strands of the sequence shown in SEQ ID NO:1.

8.   An isolated human Bad polypeptide, comprising substantially the amino acid sequence shown in SEQ ID NO:2, or functional fragment thereof.

9.   The isolated human Bad polypeptide of claim 8, wherein said functional fragment further comprises the Bcl-X$_L$ binding domain.

10.   A method of identifying a human Bad binding partner, comprising contacting human Bad or a functional fragment thereof with a sample suspected of containing a human Bad binding partner and determining the presence of binding.

11.   The method of claim 10, wherein said binding is determined *in vitro*.

12.   The method of claim 10, wherein said binding is determined *in vivo*.

13.   The method of claim 12, wherein said binding is determined by the detection of a reporter gene.

14.   The method of claim 10, wherein said functional fragment thereof is a Bcl-X$_L$ binding domain.

15.   The method of claim 10, wherein said binding partner is a human Bad interacting protein.

16.   A method of screening for a compound which interferes with the association of a human Bad interacting polypeptide with human Bad, comprising contacting human Bad, or a functional fragment thereof in the presence of an interacting polypeptide with a sample suspected of containing a compound capable of interfering with the human Bad polypeptide association and determining the interaction between human Bad and human Bad interacting polypeptide.

17.   The method of claim 16, wherein said interaction is a binding interaction.

18.   The method of claim 17, wherein said binding is determined *in vitro*.

19.   The method of claim 17, wherein said binding is determined *in vivo*.

20.   The method of claim 19, wherein said binding is determined by the detection of a reporter gene.

21.   The method of claim 16, wherein said functional fragment thereof is a Bcl-X_L binding domain.

22.   A method of decreasing the viability of a cell characterized by decreased programmed cell death comprising introducing into the cell an effective amount of human Bad or functional fragment thereof.

23.   The method of claim 22, wherein said introducing comprises, expressing a nucleic acid encoding human Bad or a functional fragment thereof.

24.   A method of increasing the viability of a cell characterized by increased programmed cell death, comprising introducing into the cell an effective amount of a compound which inhibits binding of human Bad.

25.   The method of claim 24, wherein said introducing comprises, expressing a nucleic acid encoding a polypeptide capable of binding to a human Bad binding domain.

26. The method of claim 24, wherein said compound binds to the Bcl-X_L binding domain of human Bad and prevents binding of human Bad to Bcl-X_L.

27. The method of claim 24, wherein said compound binds to the human Bad binding domain of Bcl-X_L and prevents binding of human Bad to Bcl-X_L.
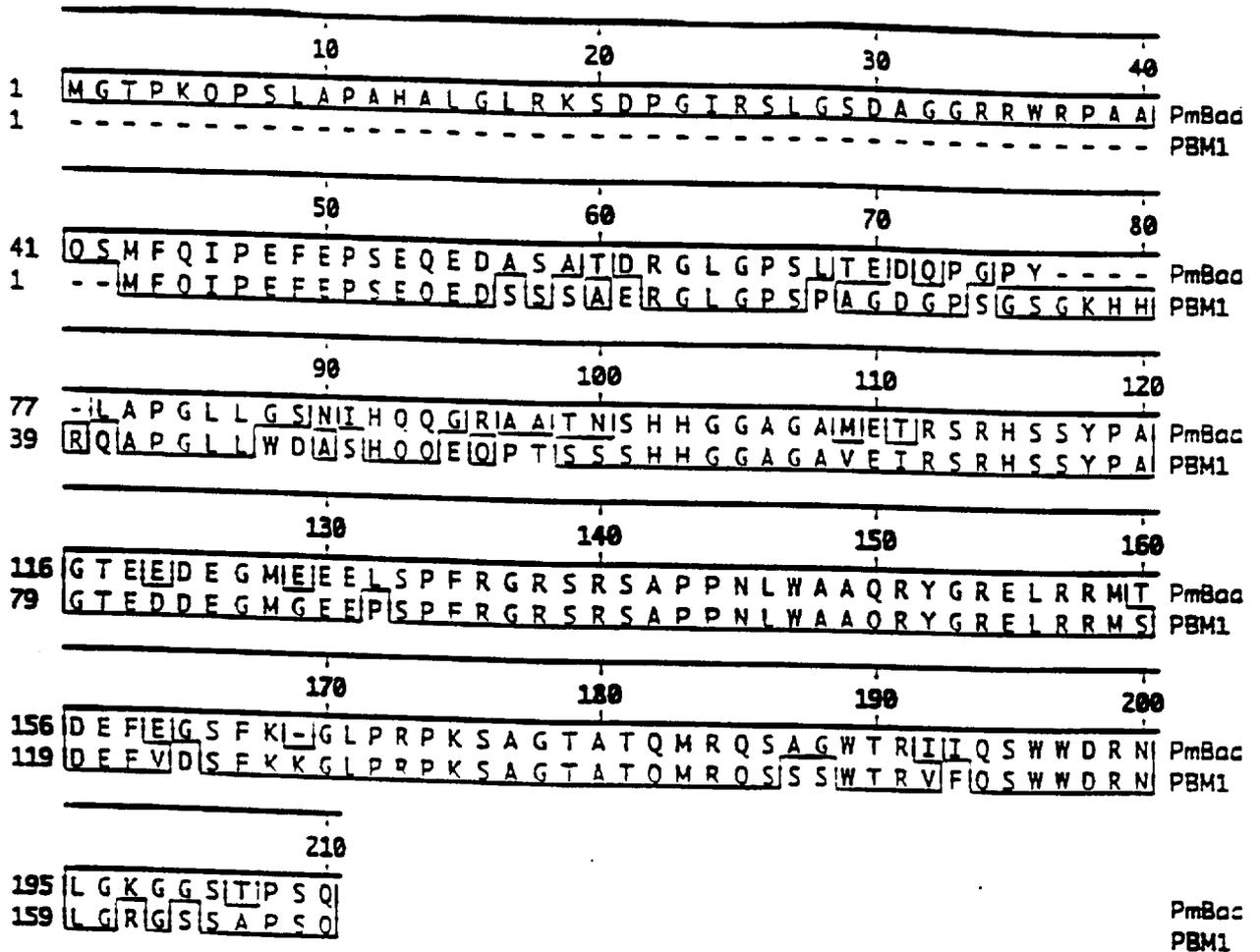
28. The method of claim 24, wherein said compound is selected from the group consisting of small molecules, peptides and peptide mimetics.

29. The method of claim 24, wherein said compound inhibits a post-translational modification of human Bad.

FIGURE 1

```
GGGCCTAGGG CGCCGGGTCA GGGGCCTCGA GATCGGGCTT GGGCCCAGAG C ATG TTC      57
                                                          Met Phe
                                                            1
```

```
CAG ATC CCA GAG TTT GAG CCG AGT GAG CAG GAA GAC TCC AGC TCT GCA      105
Gln Ile Pro Glu Phe Glu Pro Ser Glu Gln Glu Asp Ser Ser Ser Ala
          5               10                  15
```

```
GAG AGG GGC CTG GGC CCC AGC CCC GCA GGG GAC GGG CCC TCA GGC TCC      153
Glu Arg Gly Leu Gly Pro Ser Pro Ala Gly Asp Gly Pro Ser Gly Ser
    20              25                  30
```

```
GGC AAG CAT CAT CGC CAG GCC CCA GGC CTC CTG TGG GAC GCC AGT CAC      201
Gly Lys His His Arg Gln Ala Pro Gly Leu Leu Trp Asp Ala Ser His
35              40                  45                      50
```

```
CAG CAG GAG CAG CCA ACC AGC AGC AGC CAT CAT GGA GGC GCT GGG GCT      249
Gln Gln Glu Gln Pro Thr Ser Ser Ser His His Gly Gly Ala Gly Ala
                55              60                  65
```

```
GTG GAG ATC CGG AGT CGC CAC AGC TCC TAC CCC GCG GGG ACG GAG GAC      297
Val Glu Ile Arg Ser Arg His Ser Ser Tyr Pro Ala Gly Thr Glu Asp
            70                  75                  80
```

```
GAC GAA GGG ATG GGG GAG GAG CCC AGC CCC TTT CGG GGC CGC TCG CGC      345
Asp Glu Gly Met Gly Glu Glu Pro Ser Pro Phe Arg Gly Arg Ser Arg
            85                  90                  95
```

```
TCG GCG CCC CCC AAC CTC TGG GCA GCA CAG CGC TAT GGC CGC GAG CTC      393
Ser Ala Pro Pro Asn Leu Trp Ala Ala Gln Arg Tyr Gly Arg Glu Leu
    100             105                 110
```

```
CGG AGG ATG AGT GAC GAG TTT GTG GAC TCC TTT AAG AAG GGA CTT CCT      441
Arg Arg Met Ser Asp Glu Phe Val Asp Ser Phe Lys Lys Gly Leu Pro
115             120                 125                 130
```

```
CGC CCG AAG AGC GCG GGC ACA GCA ACG CAG ATG CGG CAA AGC TCC AGC      489
Arg Pro Lys Ser Ala Gly Thr Ala Thr Gln Met Arg Gln Ser Ser Ser
            135                 140                 145
```

```
TGG ACG CGA GTC TTC CAG TCC TGG TGG GAT CGG AAC TTG GGC AGG GGA      537
Trp Thr Arg Val Phe Gln Ser Trp Trp Asp Arg Asn Leu Gly Arg Gly
            150                 155                 160
```

```
AGC TCC GCC CCC TCC CAG TGACCTTCGG TCCACATCCC GAAATCCACC           585
Ser Ser Ala Pro Ser Gln
            165
```

```
CGTTCCCATT GCCCTGGGCA GCCATTTTGA ATATGGGAGG AAGTAAGTTC CCTCAGGCCT   645

ATGCAAAAAG AGGATCCGTG CTGTATCCTT TGGAGGGAGG GTTGACCCAG ATTCCCTTCC   705

GGTGTGTGTG AAGCCACGGA AGGTTGGTCC CATCGGAAGT TTTGGGTTTT CCGCCCACAG   765

CCGCCGGAAG TGGCTCCGTG GCCCCGCCCT CAGGTTCCGG GGTTTCCCCC AGGCGCCTGC   825

GCTAAGTAGC GAGCCAGGTT TAACCGTTGT GTCACCGGGA CCCGAGCCCC CGCGATGCCC   885

TGGGGGCCGT GATCAGTACC AAATGTTAAT AAAGCCCGCG TGTGTGCCAA AAAAAAAAA    945
A
                                                                    946
```

ALIGNMENT OF MOUSE AND HUMAN BAD PROTEIN SEQUENCES

```
                    10               20               30               40
1   M G T P K Q P S L A P A H A L G L R K S D P G I R S L G S D A G G R R W R P A A   PmBad
1   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   PBM1


                    50               60               70               80
41  Q S M F Q I P E F E P S E Q E D A S A T D R G L G P S L T E D Q P G P Y - - - -   PmBad
1   - - M F Q I P E F E P S E Q E D S S S A E R G L G P S P A G D G P S G S G K H H   PBM1


                    90              100              110              120
77  - L A P G L L G S N I H Q Q G R A A T N S H H G G A G A M E T R S R H S S Y P A   PmBad
39  R Q A P G L L W D A S H Q Q E Q P T S S S H H G G A G A V E T R S R H S S Y P A   PBM1


                   130              140              150              160
116 G T E E D E G M E E E L S P F R G R S R S A P P N L W A A Q R Y G R E L R R M T   PmBad
79  G T E D D E G M G E E P S P F R G R S R S A P P N L W A A Q R Y G R E L R R M S   PBM1


                   170              180              190              200
156 D E F E G S F K - G L P R P K S A G T A T Q M R Q S A G W T R I I Q S W W D R N   PmBad
119 D E F V D S F K K G L P R P K S A G T A T Q M R Q S S S W T R V F Q S W W D R N   PBM1


                   210
195 L G K G G S T P S Q                                                              PmBad
159 L G R G S S A P S Q                                                              PBM1
```

PmBad =   mouse protein sequence

PBM1=   human protein sequence

Figure 2

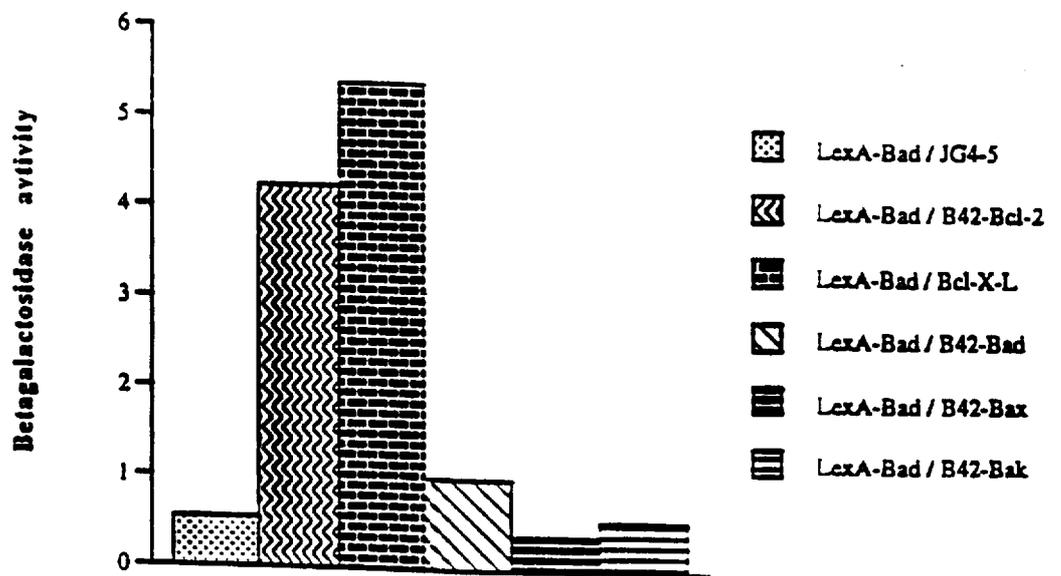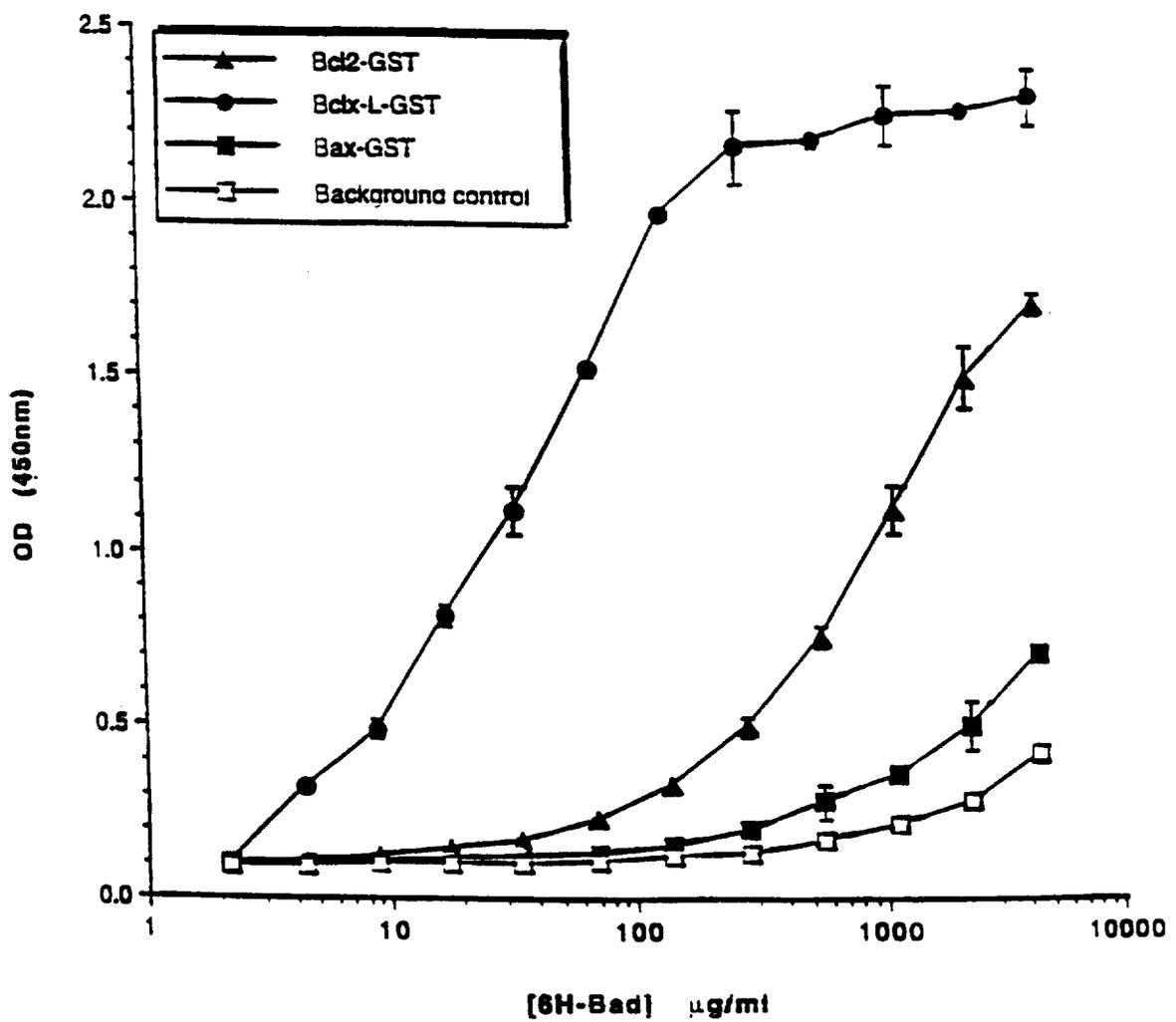## Bad 2-Hybrid Interactions

Figure 3

Figure 4