Bcl-2 homologous antagonist killer

Product specification

Acronym: hBak
Class: Transporter
Origin: Human
Molecular weight: 17 kDa
Application: Protein delivery

Purity: >75%
Activity: Proven
Length: Truncated form
TMD: 1
Biological function: Pro-apoptotic

Product description

Human Bak (hBak) is a multidomain proapoptotic member located in the outer mitochondrial membrane (OMM), containing a unique transmembrane domain located on the C-terminus part of the protein. Bak is a key regulator of programmed cell death and controls apoptosis through protein-protein interaction. It is a member of the Bcl-2 family of antiapoptotic and proapoptotic proteins.

Protein Source: hBak with a deletion of the first 70 N-terminus amino acids
Fig.1: AA sequence of hBak protein

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          10         20         30
MSGSHHHHHH SSGIEGRGRL IKHPEMVTLP
          40         50         60
LQPSSTMGQV GRQLAIIGDD INRRYDSEFQ
          70         80         90
TMLQHLQPTA ENAYEYFTKI ATSLFESGIN
         100        110        120
WGRVVALLGF GYRLALHVYQ HGLTGFLGQV
         130        140        150
TRFVVDFMLH HCIARWIAQR GGWVAALNLG
         160        170     177
NGPILNVLVV LGVVLLGQFV VRRFFKS
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Affinity Tag: Histidine tag fused to the N-terminal end of the protein.

Production conditions: hBAK is expressed in a cell-free expression system in the presence of lipid vesicles. 100 µg can be produced and qualified in about 1 week

Quality analysis

Purity: Typically >75% as determined by SDS-Page and Coomassie Blue staining.

Purification procedure: As standard, hBak proteoliposomes are purified on a sucrose gradient. Further purification steps can be added if required.
Activity: Recombinant proteoliposomes containing hBAK have been shown to be fully active in in vitro, in cellulo and in vivo experiments and in cellular internalization.

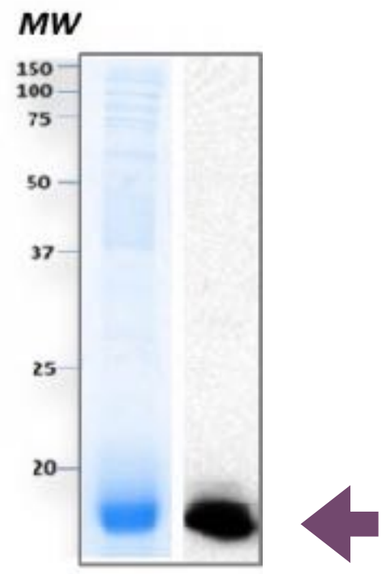

Fig.2: hBak proteoliposome after purification (Coomassie Blue quantification and Western blot identification).

Assessment of functionality

Cell-free expression systems provide a real alternative for membrane protein expression, enabling the study of structure and function of membrane proteins.

Methods: in vitro; in cellulo; in vivo tests

The Human Bak protein was expressed in Synthelis' cell-free system in the presence of liposomes to obtain proteoliposomes in a one-step reaction. The apoptotic effect of the hBak proteoliposomes is confirmed in cellulo onto glioblastoma cells by activation of intrinsic caspase pathway and in vivo in glioblastoma murine model by induction of a total tumor regression.

Results:

In vitro pro-apoptosis activity

Release of cytochrome C, caspase 7, caspase 3, caspase 9 and PARP activation were identified by Western Blot performed on the supernatant from the centrifugation of lysed mitochondria treated with an increasing amount of proteoliposomes.

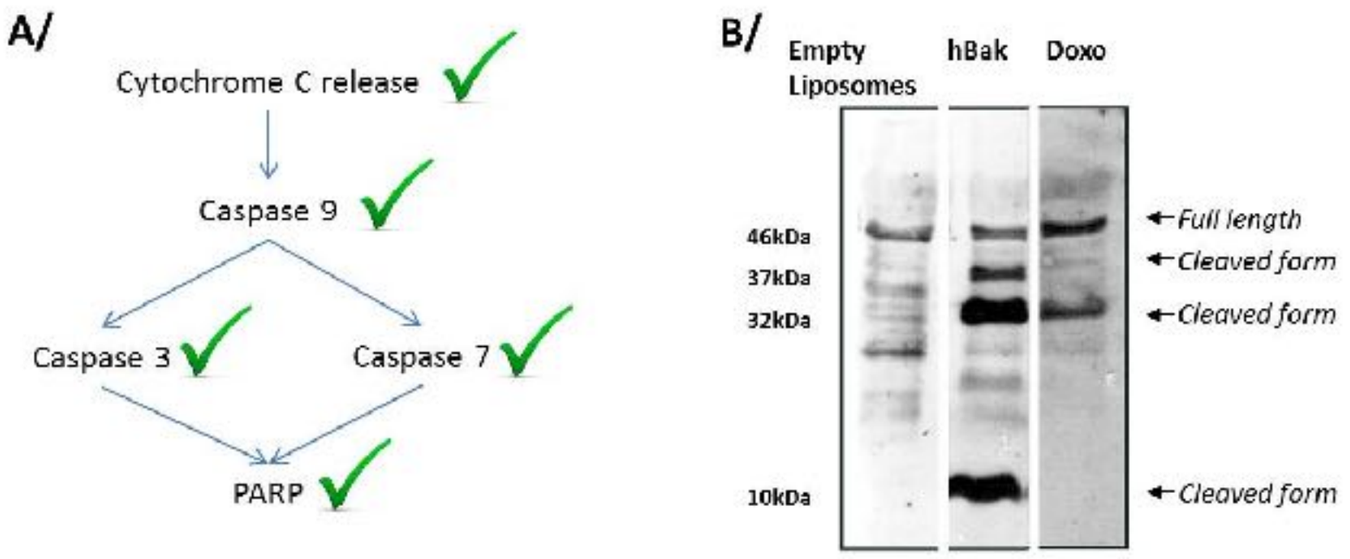

Fig.3: In vitro pro-apoptotics activity. A/ Intrinsic apoptosis pathway activated by hBak proteoliposomes treatment. B/ Western Blotting analysis reveals the caspase 9 activation on glioblastoma cells by hBak proteoliposomes treatment.

In cellulo

During the early apoptotic phase, recombinant hBak was localized with mitochondria, while after 24h hBak was concentrated in the mitochondria membranes. This result proved that the proteoliposomes are able to deliver a functional recombinant protein directly into cells (Liguori, and al., 2008). hBak proteoliposomes (+/- pegylated) vectorized into cells are able to induce apoptotic cell death.

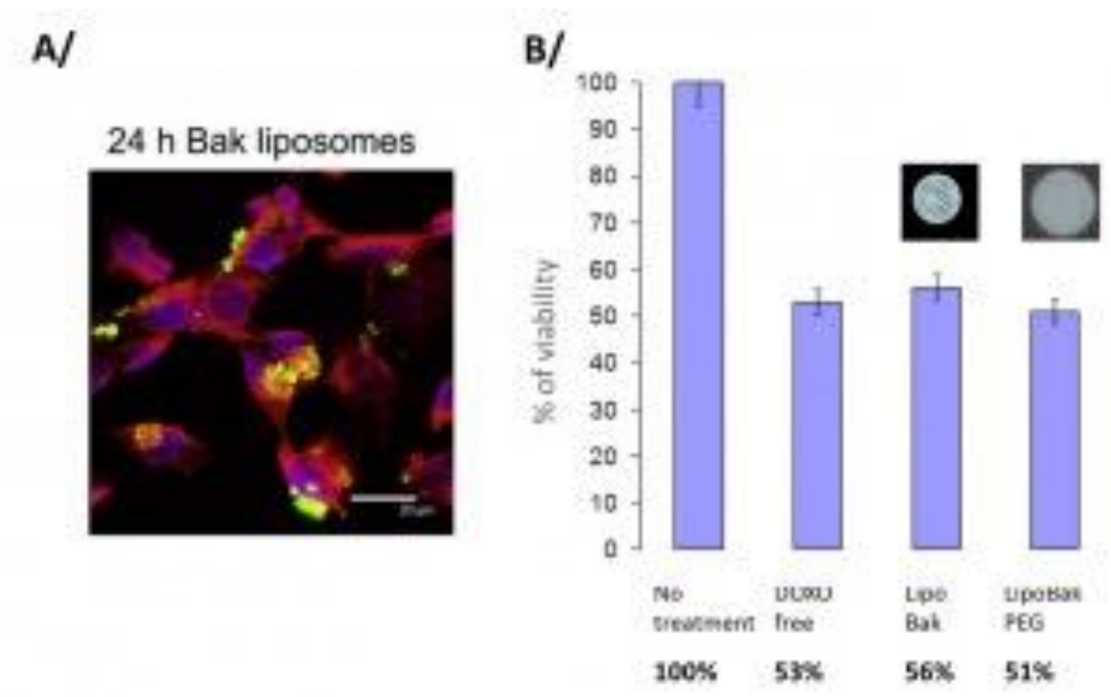

Fig.4: In vitro pro-apoptotic activity. A/Immunofluorescence on cells incubated with hBak proteoliposomes. B/ MTT cell viability assay after addition of hBak proteoliposomes (+/- pegylated). Doxo (Doxorubicin) was used as a positive control.

In vivo pro-apoptotic activity

Tumor-bearing mice were treated by intratumoral injections of hBak proteoliposomes or empty liposomes. After the end of the treatment, while in the presence of empty liposomes the mice survive only a few days with an incredible increase of tumor size, in the presence of hBak proteoliposomes 60% of the mice survive showing a total tumor disappearance.

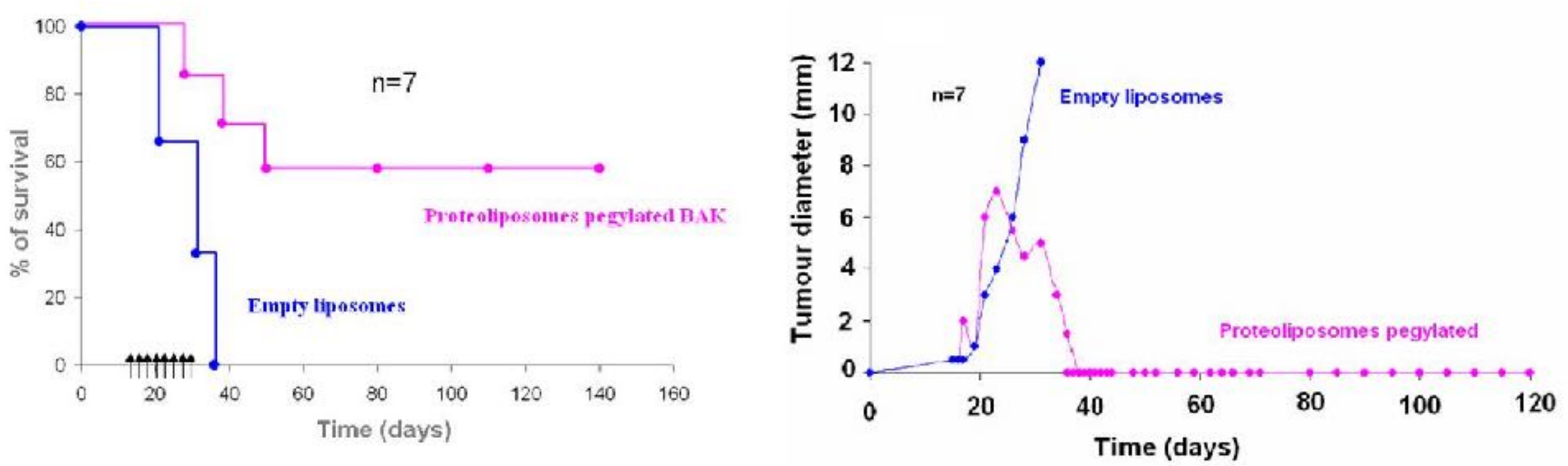

Fig.5: In vivoanti-tumoral activity of recombinant hBak proteoliposomes. A/ Survival curves. B/ tumor growth in tumor-bearing mice. Mice were subcutaneously injected with glioblastoma cells at day 0 then treated with empty liposomes or hBak proteoliposomes. The capacity to survive and the evolution of the tumor size were analyzed after the end of medication treatment.

Quality Control

Cell-free expression system in the presence of liposomes is an efficient method to produce Bak recombinant proteoliposomes. Cross-linking assays using photoactivable amino acid analogs demonstrate the oligomeric states into the liposomes, which is essential for the pro-apoptotic function.

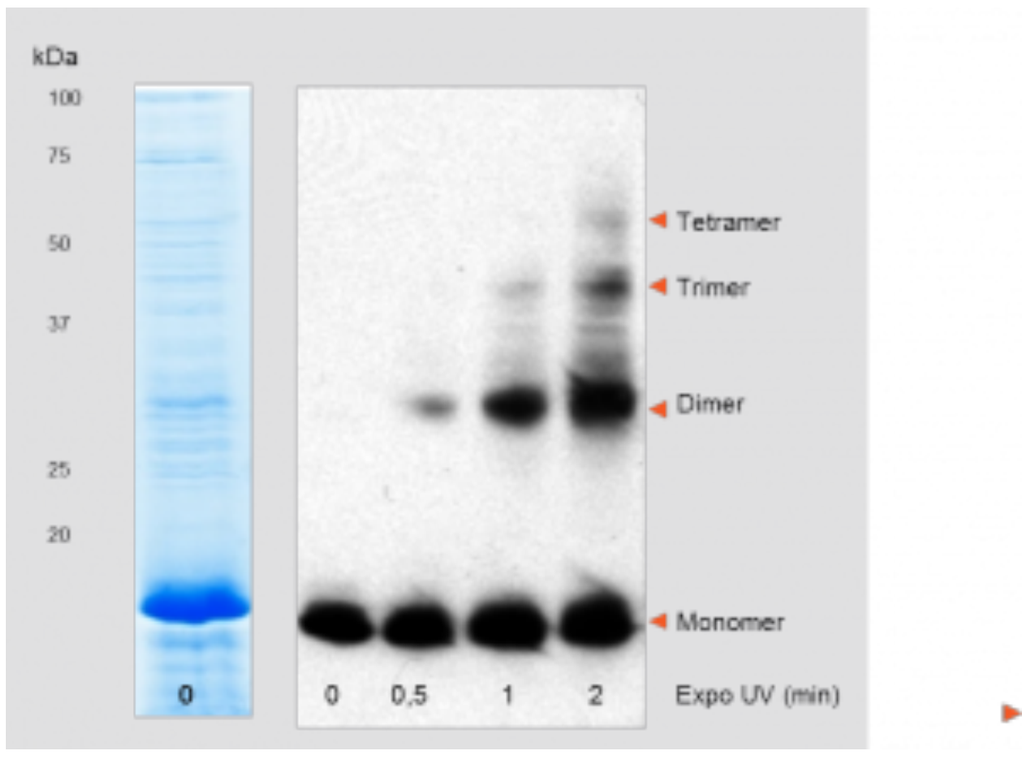

Fig.6: Cross-linking assays of hBak in liposome membranes using photoactivable amino acid analogs. Immunoblotting analysis of hBak resolved by SDS-PAGE. Samples were exposed to UV light for different exposure times.

Formulation

Buffer: Available in PBS, Tris 50mM, pH 7.5, HEPES 50mM, pH 7.5. Others buffers or customized formulation can be provided upon request

Customized Hydrophobic matrix: Customized formulation with specific lipids like PEGylated or biotinylated lipids can be used upon request, as well as targeting molecules.

Storage/Stability: Store at +4°C for up to one week or several months at -80°C. Aliquot for storage. Do not freeze-thaw after aliquoting.

Use restrictions: For life science research use only.

Available sizes: 25µg ,100 µg ,500 µg,bulk

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