GRANULYSIN, OBTENTION METHOD AND USES

Spanish Patent

DESCRIPTION:

Granulysin is a naturally occurring protein that plays a key role in the immune response. This invention describes a novel method to obtain Granuliysin and its use as a systemic anticancer drug.

It has been described that the 9 kDa isoform of recombinant Granulysin is cytotoxic on tumor cells. By using the DNA recombinant technique, the researchers have been able to express the 9kD isoform in *Pichia Pastoris* (Fig. 1), obtaining high yields with no lipopolysaccharide content, and purified using affinity chromatography (Fig.2). After purification, the inventors demonstrated that the chimeric protein retains its functionality

At a later stage, the chimeric protein was linked to the small chain fragment variable (scFv) of the anti-CEA antibody MFE23 (Fig 3).

Finally, experiments have been successfully conducted to validate IN VIVO Granulysin anti-tumor activity (Fig.4).

Fig. 1 Granulysin expresion in Picchia Pastoris

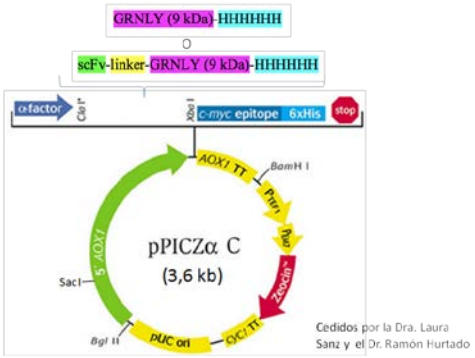
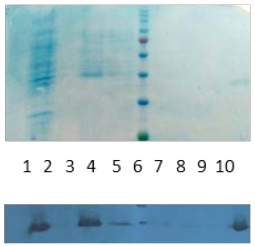


Fig. 2 GRNLS Purification



Granulysine 9kDa

"Different functionalizations are possible, expanding the type of tumor to be treated"

VALIDATION TEST:

IN VITRO and IN VIVO trials have been successfully carried out for several types of Cancer. Experimental results show that 9 kDa Granulysin expressed in *P. Pastoris* has higher cytotoxicity than Granulysin expressed in *E. Coli*

- Jurkat Cells: To obtain 90% of toxicity it is required concentrations around 50 µM of Granulysin expressed in *E. coli* or around 15 µM of Granulysin expressed in *P. pastoris*.

- HT29 Cells: To obtain 60% of toxicity, it is required a concentration of 10 µM of Granuliysin expressed in *P. Pastoris*.

In both cases, when Granuliysin is in the chimeric form, the same level of cell death is obtained just with a concentration around 6 µM.

Researchers have demonstrated that Chimeric Granuliysin obtained by this patented method is more effective than no chimeric Granulysin, whether on solid tumors (i.e. colon) or on leukemia.

"Systemic administration of Chimeric Granulysin has been validated as an antitumor agent in the treatment of cancer"

"By means of systemic administration, Granulysin is able to reach the tumor with enough concentration to display its cytotoxic activity"

EXPERIMENT WITH NUDE MICE WITH TUMORS FROM HT29

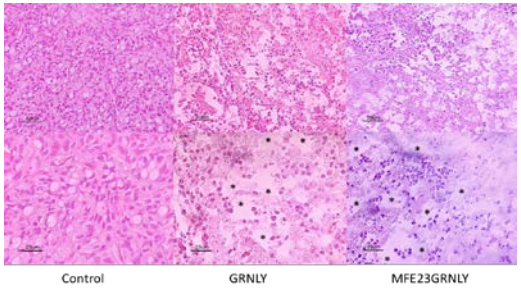
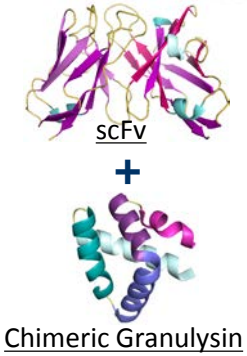


Fig. 3 **scFv Functionalization**

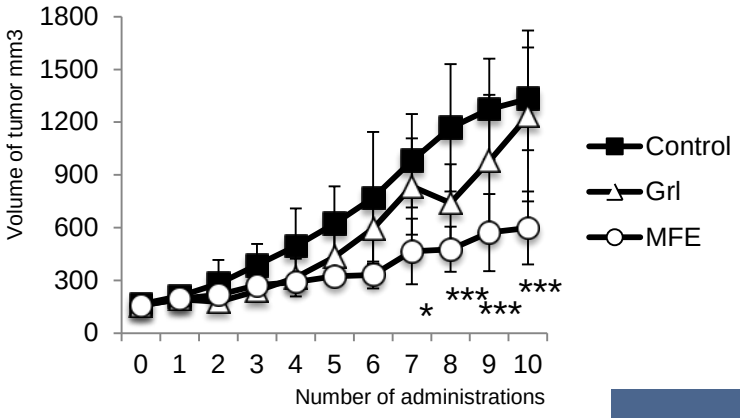


"Treatment IN-VIVO validated"

INNOVATIVE FEATURES:

1. Granuliysin is obtained following a novel Method based on *P. Pastoris*, which allows to obtain the 9kD Isoform, cytotoxic against tumor cells.
2. By this method the presence of LPS is eliminated.
3. Granulysin has been successfully functionalized with several specific scFv of Antibodies, that recognize different tumor cells, making the treatment highly effective.
4. Functionalized Granulysin may be applied on a variety of solid tumors.

Fig.4 **IN VIVO Experiments**



Tumor model of HeLa-CEA cells in atimic mice

COLLABORATION THE UNIVERSITY OF ZARAGOZA

University of Zaragoza aims to introduce this Cancer Treatment into the market, so is pleased to initiate contacts with those biotech and pharma companies who are interested in the commercial exploitation of this treatment.

Together we will explore all possible scenarios to cooperate on developing this invention. We follow a win-win strategy with our industrial partners.

If you require further information about this treatment, please contact our technology transfer office (TTO) in the address below:

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