ELECTRONIC SUPPLEMENTARY MATERIAL

Rössler-Fehr V *et al.*: Effects of sevoflurane on natural killer cell-induced apoptosis of malignant tumour cells: an *in vitro* laboratory study

Disclaimer: *Electronic Supplementary Material (ESM) is supplied by the authors for the benefit of readers. This feature is intended to provide additional information, context, and/or dimension to complement articles. Readers should be aware that unlike the main print manuscript, ESM may not undergo extensive peer review and typically is posted as supplied by the authors without editing, proofreading, accuracy checking, copyediting, or typesetting; it is published online only.*

TABLE OF CONTENTS

eAppendix Additional materials and methods

Flow cytometry

Cells were washed with phosphate-buffered saline (PBS) (Mediatech, Manassas, VA, USA). The surface was stained with PE-CD56 (BD Biosciences, San Jose, CA, USA) as CD56 is expressed on the surface of NK92 cells. Simultaneously, viability was determined by staining with FVS660 (BD Horizon, San Jose, CA, USA), followed by washing in annexin V binding buffer and annexin V (AV) immunostaining (both from Biolegend, San Diego, CA, USA). Viable cells are FVS660 negative (FVS660-), necrotic cells are FVS660+. Apoptotic cells are AV positive (AV+), non-apoptotic cells are AV-. An overview of the antibodies used can be found in **Table S1**.

In a second assay, NK92 degranulation was measured. The pre-treatment period with sevoflurane, cell ratio, and co-incubation time was chosen as described, and only a co-incubation time of 4h was evaluated. FITC-CD107a (BD Biosciences) was added. After 1 hour, 50 mM Monensin (Sigma, St. Louis, MO, USA) was added to prevent CD107a antibody degradation. Cells were then washed with PBS, and surface staining with PE-CD56 and FVS660 was performed. The following controls were included: unstimulated NK92 cells (negative control) and NK92 cells stimulated with 50 nm Phorbol 12-myristate 13-acetate (PMA) (Calbiochem, San Diego, CA, USA) and 1 mM Ionomycin (Sigma) (positive control).

Cells were fixed with 1 % paraformaldehyde for the first and second assays and analyzed on an LSR Fortessa flow cytometer (BD Biosciences). Fluorescence minus one (FMO) control was included in both experiments to set gates. For the degranulation assay, the unstimulated control was used to detect baseline degranulation, and gates were set accordingly. FlowJo V 10 software (FlowJo, LCC, Ashland, OR, USA) was used to analyze the data.

Table S1: Antibody details for flow cytometry

Flow cytometry	Annexin V	Biolegend, cat. # 640905
	FVS660	BD Horizon, cat # 564405
	PE-CD56	BD Biosciences, cat # 555516
	FITC-CD107a	BD Biosciences, cat # 555800
Western blot	GAPDH	Santa cruz, sc-25778
	Perforin	Santa cruz, sc-373943
	LAMP-1	Santa cruz, sc-18821

Quantitative real-time polymerase chain reaction analysis

Trizol (Invitrogen, Carlsbad, CA, USA) was used for total RNA isolation. The RNA concentration was determined with a Nanodrop analyzer (Thermo Fisher Scientific, Hampton, NH, USA). Subsequently, 1200 ng of RNA was reverse transcribed to cDNA using the thermal cycler T100 (Bio-Rad Laboratories, Hercules, CA, USA) and a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) according to manufacturer's instructions. Real-time reverse-transcription polymerase chain reaction (rt-PCR) with Fast SYBR master mix kit and an ABI 7500 FAST system (both from Applied Biosystems) was performed.

The primers used can be found in **Table S2**. As described previously, cycle threshold (Ct) values were analyzed, and glyceraldehyde3-phosphate-dehydrogenase (GAPDH) was used as an amplification control for each sample. Relative mRNA levels of the groups were calculated, whereby the co-incubation group in which NK92 cells had been exposed to "air" served as the reference value.

Table S2: Polymerase chain reaction (PCR) primer details

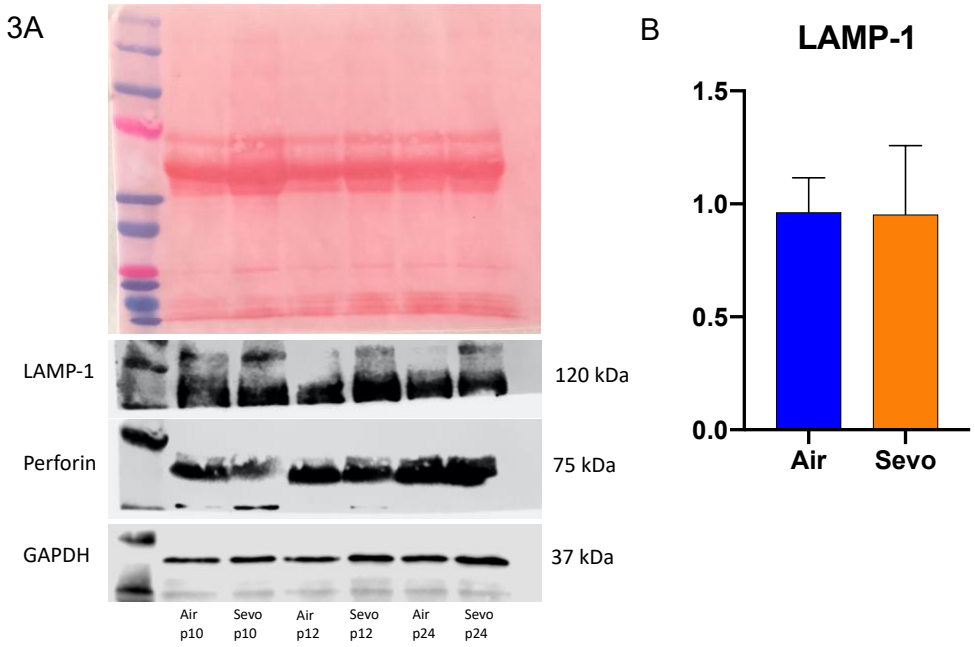
GADPH	Forward primer 5'-ggg tgt gaa cca cga gaa ata-3' Reverse primer 5'-gtc atg agc cct tcc aca at-3'
IFN- γ	Forward primer 5'-gtg gag acc atc aag gaa gac-3' Reverse primer 5'-acc tcg aaa cag cat ctg ac-3
IL-6	Forward primer 5'-cga gag tcc ttc aga gag ata ca-3' Reverse primer 5'-cct tct gtg act cca gct tat c-3'
TNF-α	Forward primer 5'-cga tgg gtt gta cct tgt cta c-3' Reverse primer 5'-gca gag agg agg ttg act ttc-3'

GADPH = glyceraldehyde 3-phosphate dehydrogenase; IFN-γ = interferon-gamma;
IL-6 = interleukin-6; TNF-α = tumor necrosis factor-alpha

Enzyme-linked immunosorbent assay (ELISA)

The cell pellet and the supernatant were collected for perforin protein measurement. Supernatants were processed directly, and cell pellets were lysed in 2 ml PBS supplemented with 20 ml protease inhibitor cocktail (#P8340, DMSO Solution Sigma-Aldrich). To promote the breaking of cell walls, a Branson sonifier (Cell Disruptor B15, SKAN AG, Basel, Switzerland) was used. The settings were: 10% duty cycle, continuous pulse, output con 7. Thereafter, samples were centrifuged at 2,190 × *g* for 5 min. Supernatants were collected for intracellular perforin detection; cell debris was discarded. For perforin quantification, an ELISA kit from Abcam (ab83709 – perforin human ELISA set) was used according to the supplied protocol. Concentrations of perforin were measured in diluted supernatants (1:250) and cells (1:500). Protein concentrations were corrected for cellular DNA content. Assays were performed in triplicate.

eFigure LAMP-1 determination in EVs. Western blots were performed to analyze the expression of LAMP-1 as a marker of the origin of the EVs and perforin expression (A). Both untreated (air) and sevoflurane treated (sevo) cells showed the same LAMP-1 expression (B). Analysis was performed with unpaired *t* tests. Values represent means (standard deviations). Three experiments were included in the analysis.



EV = extracellular vesicle; GADPH = glyceraldehyde 3-phosphate dehydrogenase; LAMP-1 = lysosomal-associated membrane protein-1