

## Cytotoxic reactions of CC531s towards liver sinusoidal endothelial cells: a microscopical study

Katrien Vekemans<sup>\*1</sup>, Maarten Timmers<sup>1</sup>, David Vermijlen<sup>1</sup>, Ronald De Zanger<sup>1</sup>, Eddie Wisse<sup>1</sup> and Filip Braet<sup>1,2</sup>

Address: <sup>1</sup>Laboratory for Cell Biology and Histology, Free University of Brussels (VUB), Laarbeeklaan 103, 1090 Brussels, Belgium and <sup>2</sup>Present address: Department for Molecular Biomedical Research, Molecular Cell Biology Unit, Ghent University (UGhent), Technologiepark 927, 9052 Zwijnaarde, Belgium

Email: Katrien Vekemans<sup>\*</sup> - kvekeman@cyto.vub.ac.be; Maarten Timmers - mtimmers@cyto.vub.ac.be; David Vermijlen - dvermijl@cyto.vub.ac.be; Ronald De Zanger - ronald@cyto.vub.ac.be; Eddie Wisse - wisse@minf.vub.ac.be; Filip Braet - filipbra@minf.vub.ac.be

<sup>\*</sup> Corresponding author

from 11th International Symposium on the Cells of the Hepatic Sinusoid and their Relation to Other Cells  
Tucson, Arizona, USA, 25–29 August, 2002

Published: 14 January 2004

*Comparative Hepatology* 2004, **3**(Suppl 1):S49

This article is available from: <http://www.comparative-hepatology.com/content/3/S1/S49>

### Introduction

Colorectal cancer cells can induce apoptosis in cells of various tissues [1]. Apoptosis can be induced by a number of factors such as Fas inducing apoptosis through the Fas/FasL pathway; other factors involve the TRAIL pathway and TNF. It is known that metastasizing colon cancer cells express more FasL than primary carcinoma cells [2]. We investigated whether a rat colon carcinoma cell line CC531s could induce apoptosis in liver sinusoidal endothelial cells (LSECs). LSECs and CC531s were co-cultured for 18 hrs and cells were visualized by SEM and TEM. Apoptosis was visualized by markers such as Hoechst and Propidium iodide. Furthermore, cells were recorded by time lapse video microscopy with and without an antagonistic antibody for FasL.

### Methods

#### Animals, antibodies & reagents

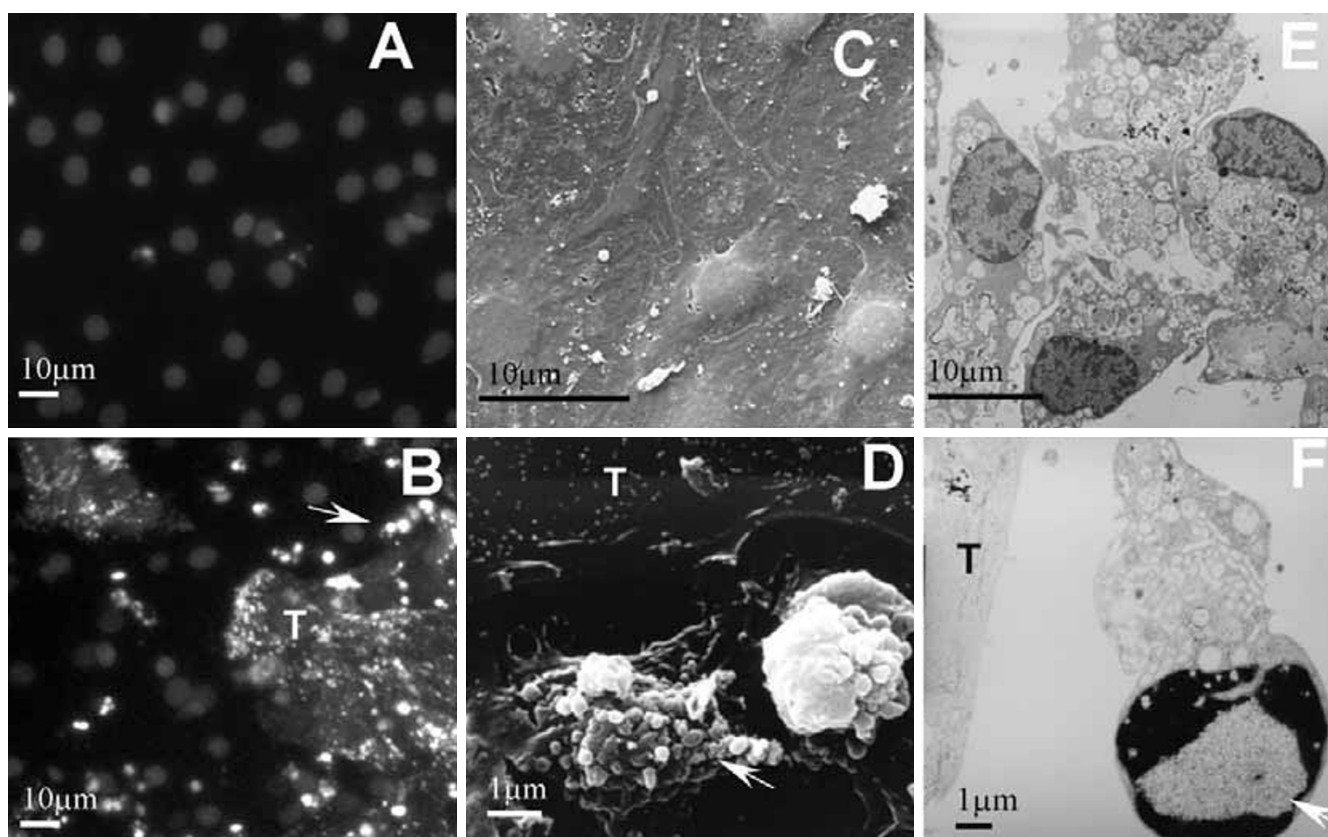
Male Wistar rats (8–12 weeks old) were purchased at the Center of Laboratory Animals (Leuven, Belgium). Animals had free access to food and water. 3,3'-diiodo-4,4'-dimethoxy-5,5'-diphenylsulfone (DiO) (catalogue no. D-275), Hoechst 33342 (HO 33342) (Catalogue no. H-3570) and Propidium iodide (PI) (catalogue no. P-3566) were purchased at Molecular Probes (Leiden, The Netherlands). Anti-FasL blocking antibody, MFL-4, was obtained from BD Pharmingen (Erembodegem, Belgium, catalogue no. 555021).

#### Co-culture of CC531s cells and LSECs

LSECs were isolated [3], purified and seeded to confluence on collagen-S coated wells and RPMI- 1640 supplemented with 10% heat inactivated fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin and 100 micrograms/ml streptomycin. The cells were kept in culture for 4 hrs. Subsequently, cells were washed with prewarmed RPMI. CC531s cells were seeded on top of LSECs at a ratio of 1:10. In order to visualize apoptosis, cells were stained with Hoechst 33342 and propidium iodide [4]. In order to discriminate cells, CC531s were stained with DiO [5]. For TEM and SEM, cells were prepared [6] and viewed with F. E. I. Tecnai 10 and Philips SEM 505. For time lapse recordings, cells were recorded up to 1 hr. Cells were cultured in medium only and/or with an antagonistic antibody against FasL (20 micrograms/ml).

### Results and Discussion

After 18 hrs of co-culture of LSECs and CC531s, LSECs reveal typical features of apoptosis (Fig. 1). After Hoechst and propidium iodide staining LSECs neighboring the CC531s cells reveal fragmented nuclei, in contrast to LSECs cultures where the nuclei display a normal morphology. With electron microscopy the same observation could be made, the surface of the cells reveals blebbing and with the TEM fragmented nuclei could be observed. Both in comparison with the control condition where LSECs reveal fenestrae and sponge-like appearance of the



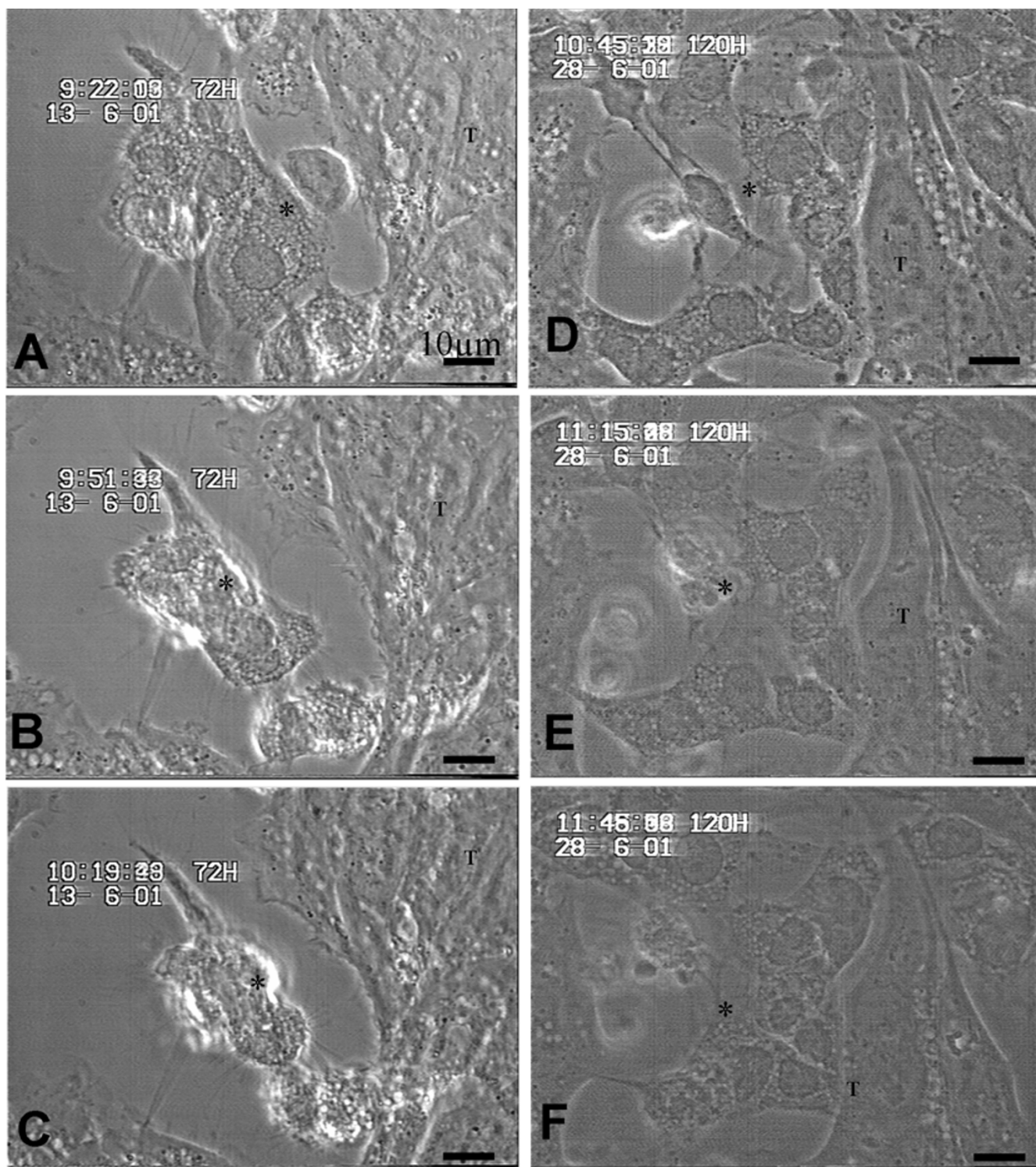
**Figure 1**

LSECs in control conditions (A, C, E) and co-culture of CC531s cells (T) and LSECs (B, D, F). A, B: Hoechst and propidium iodide staining recorded with a fluorescence microscope, Control LSECs (A) have almost no fragmented nuclei while LSECs (B) neighboring CC531s also reveal fragmented nuclei (arrow). CC531s are stained with DiO to differentiate from the LSECs. C, D: SEM recordings, in control cells (C) fenestrae can be observed and cytoplasm of the cells is well spread while CC531s neighboring the LSECs induce apoptosis in LSECs (D), as shown by blebbing and retraction of the cytoplasm (arrow). E, F: TEM recordings, control cells (E) show sponge like cytoplasm while LSECs (F) with CC531s cells show fragmented nuclei (arrow).

cytoplasm. In the literature, this type of apoptosis was also observed when human umbilical vein endothelial cells HUVEC and a mammary carcinoma cell line [7].

Time-lapse recordings (Fig. 2) provided comparable observations in the non-treated cells. At time point 0, LSECs reveal spread cytoplasm. After 30 minutes, cells start to shrink and after 1 hr cells show signs of apoptosis. In contrast, treated cells remain to have a spread cytoplasm and do not proceed to apoptosis. In accordance, colon carcinoma cell lines can also induce apoptosis in Fas bearing hepatocytes [8].

In conclusion: CC531s cells can induce apoptosis in LSECs and antagonistic antibody against FasL abrogates this apoptosis.

**Figure 2**

LSECs (\*) and CC531s (T) co-cultures were performed with (D-F) and without aFasL (A-C). After 18 hrs incubation, recordings were made for 1 hour at time points 0 (A, D), 30 (B, E) and 60 (C, F) minutes are shown here. In conditions without aFasL, cells are rounding up and reveal signs of apoptosis after contact with CC531s. In conditions with aFasL, LSECs remain unchanged.

## References

1. Pinkoski MJ, Brunner T, Green DR, Lin T: **Fas and Fas ligand in gut and liver.** *Am J Physiol Gastrointest Liver Physiol* 2000, **278**:G354-G366.
2. Mann B, Gratchev A, Bohm C, Hanski ML, Foss HD, Demel G, Trojanek B, I Schmidt-Wolf, Stein H, Riecken EO, Buhr HJ, Hanski C: **FasL is more frequently expressed in liver metastases of colorectal cancer than in matched primary carcinomas.** *Br J Cancer* 1999, **79**:1262-1269.
3. Braet F, De Zanger R, Sasaoki T, Baekeland M, Janssens P, Smedsrød B, Wisse E: **Assessment of a method of isolation, purification and cultivation of rat liver sinusoidal endothelial cells.** *Lab Invest* 1994, **70**:944-952.
4. Hoorens A, Van de Casteele M, Kloppel G, Pipeleers D: **Glucose promotes survival of rat pancreatic beta cells by activating synthesis of proteins which suppress a constitutive apoptotic program.** *J Clin Invest* 1996, **98**:1568-1574.
5. Timmers M, Vermijlen D, Vekemans K, Wisse E, Braet F: **Tracing DiO-labeled tumor cells in liver sections by confocal laser scanning microscopy.** *J Microsc* 2002, **208**:65-74.
6. Braet F, De Zanger R, Wisse E: **Drying cells for SEM, AFM and TEM by hexamethyldisilazane: a study on hepatic endothelial cells.** *J Microsc* 1997, **186**:84-87.
7. Kebers F, Lewalle JM, Desreux J, Munaut C, Devy L, Foidart JM, Noel A: **Induction of endothelial cell apoptosis by solid tumor cells.** *Exp Cell Res* 1998, **240**:197-205.
8. Yoong KF, Afford SC, Randhawa S, Hubscher SG, Adams DH: **Fas/ Fas ligand interaction in human colorectal hepatic metastases: A mechanism of hepatocyte destruction to facilitate local tumor invasion.** *Am J Pathol* 1999, **154**:693-703.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:  
[http://www.biomedcentral.com/info/publishing\\_adv.asp](http://www.biomedcentral.com/info/publishing_adv.asp)

