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# Research of diffusible factors secreted during radio-induced mammary tumor cell death

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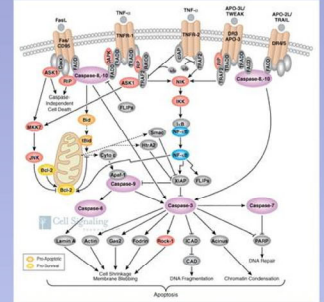
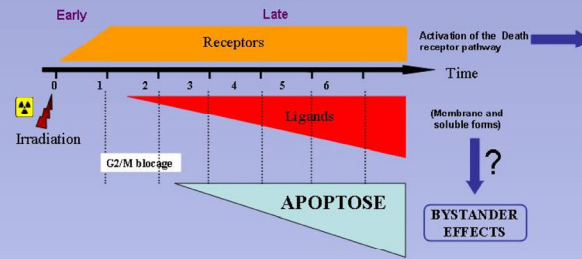
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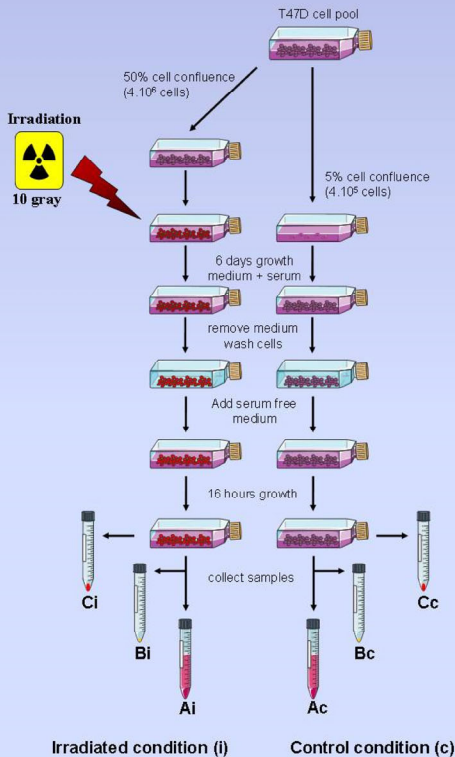
## Connexion between apoptosis and « Death receptor » pathway

A prolonged G2 blockage, followed by a mitotic disorder is considered as a frequent mode of solid tumor cell death after  $\gamma$ -irradiation. We previously showed that such delayed cell death involved mostly apoptotic mechanisms and was mediated by the Fas, TRAIL and TNF- $\alpha$  death receptor pathways through the overexpression of main ligands/receptors of these 3 pathways. Moreover we showed that the three ligands were also produced as soluble forms whose secretion can induce the death of un-irradiated cells through a "bystander" type effect.

The goal of this study is to characterize the proteins secreted by the breast cancer cell line T47D after a strong ionizing radiation treatment and to identify a large range of these diffusible factors using a global proteomic approach.



## Production of biological material



**Growth conditions:**  
T47D cells are grown in flasks in DMEM + 10% FCS  
- 4.10<sup>6</sup> cells (irradiated condition)  
- 4.10<sup>6</sup> cells (control condition)  
are transferred in new flasks

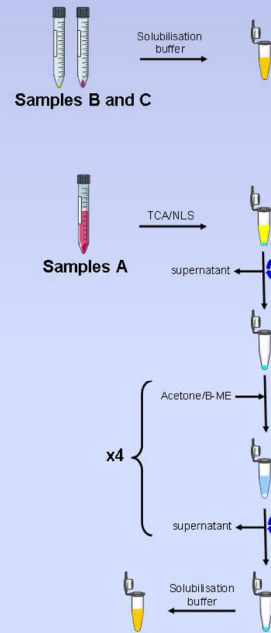
The flask containing the 4.10<sup>6</sup> cells (irradiated condition) is then submitted to a 10 gray irradiation treatment.

After a 6 days growth period in serum-complemented medium, the medium from both flasks is removed, and the adherent cells are washed several times with serum free medium

Washed cells of both conditions are then incubated during 16 hours with serum-free medium

**Samples collection:**  
**A:** medium (with secreted proteins) is aspirated from the flasks, the floating cell and debris are removed by centrifugation followed by a 0.45  $\mu$ m filtration, and the final supernatant is complemented with a protease inhibitor cocktail and kept frozen until use.  
**B:** pellet of floating cells and debris  
**C:** remaining adherent cells

## Extraction of proteins



**Samples solubilisation: B and C**  
The pellet of floating cells and debris (samples B) and the remaining adherent cells (samples C) are directly solubilized with solubilisation buffer: (7M Urea, 2M Thiourea, 4%CHAPS, 65 mM DTT, 30 mM Spermine, 0,05% TritonX100 anti-protease et anti-phosphatase cocktails). Un-soluble material and DNA are removed by ultra-centrifugation.

**Protein extraction from sample A**  
Secreted proteins are precipitated using 7.5% TCA and 0.1% NLS (sodium lauryl sarcosinate) as described by Chevalier et al. (2007)\*.

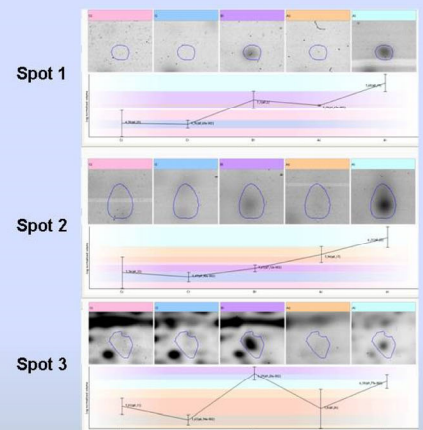
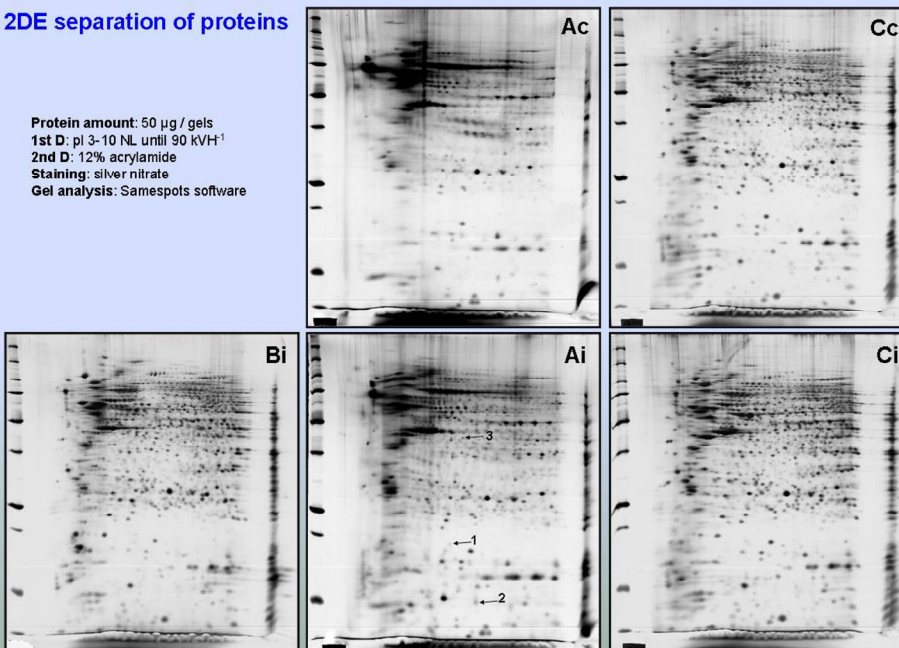
Precipitated proteins are washed 4 times using cold acetone supplemented with 0.07% B-Me as described by Chevalier et al. (2004)\* to remove 2DE incompatible compounds such as TCA and NLS.

Washed proteins are air-dried and solubilized with solubilisation buffer.

**Extraction capacity:**  
**Ai** ~60  $\mu$ g protein in 10 ml of medium  
**Ac** ~30  $\mu$ g protein in 10 ml of medium

## 2DE separation of proteins

Protein amount: 50  $\mu$ g / gels  
1st D: pI 3-10 NL until 90 KVH<sup>1</sup>  
2nd D: 12% acrylamide  
Staining: silver nitrate  
Gel analysis: Samespots software



The picture analysis using the Samespots software reveals significant differences between the control and the irradiated samples. An example of interesting spots is presented above. This preliminary study clearly shows the capacity of the selected growth conditions and proteins extraction to investigate the secreted proteins after a strong irradiation. The next step of this work will be the identification of the interesting spots using mass spectrometry.