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# Proteomic analysis of the Radiation-Induced Bystander Effects

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## Proteomic analysis of the Radiation-Induced Bystander Effects

Funded by :

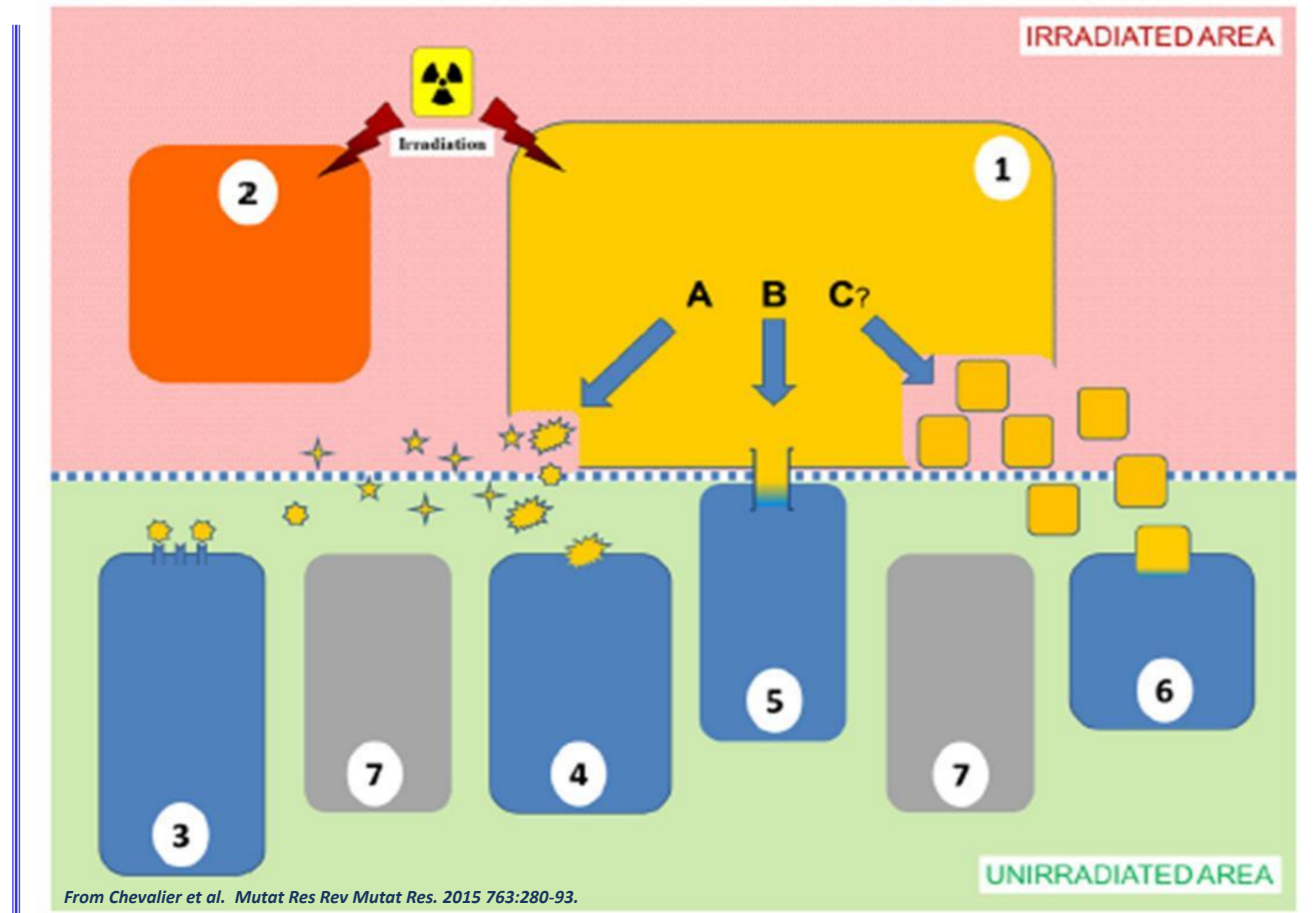


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Experimental evidence shows that, in addition to this direct and targeted effect of radiation, a side or secondary effect is observed within the un-irradiated area. Irradiated cells (1 and 2) relay a stress signal in the close proximity. Neighboring un-irradiated cells (3 to 7) react to this signal, i.e., BE with a specific "bystander response" (BR), characteristic of a biological context, but with a close relationship to the biological response typically associated with direct radiation exposure. Bystander responses and bystander factors (BF) secreted by irradiated cells were observed and studied in many physical and biological conditions, in vitro and in vivo.

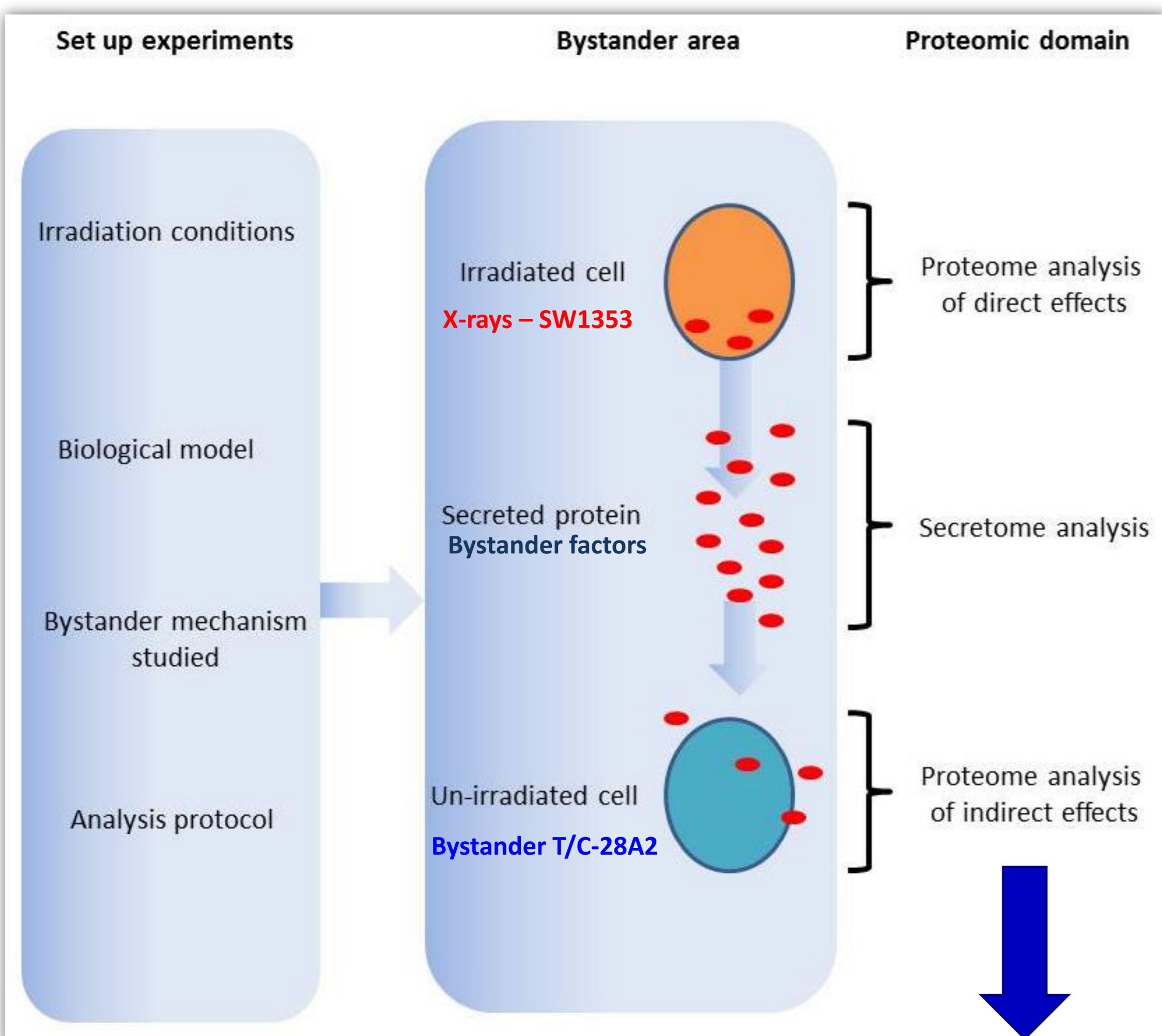
In the present study, we investigated the capacity of chondrocytes (T/C28-A2) in responding to bystander factors released by irradiated chondrosarcoma cells (SW1353) using a medium transfer protocol.



The cells were irradiated (X-rays / Carbon ions) and the Bystander supernatant was transferred on non-irradiated cells. To study the effects of these treatments on the survival of cell lines, we carried out clonogenic assays and in order to study the impact of these treatments (X-rays) on the cellular proteome, we carried out proteomic analysis by two-dimensional electrophoresis (2DE), starting from the cellular protein lysates.

The 2DE analysis showed the effect of irradiation on the proteome of irradiated chondrosarcoma cells as well as the Bystander effect on the proteome of bystander chondrocytes. We then selected variant spots that were analyzed by mass spectrometry.

An in-depth analysis of the identified proteins will provide a better understanding of the mechanisms involved in cell irradiation as well as the radio-induced Bystander effect.



### Secreted proteins : potential bystander factors

Conditioned medium (SW1353 0,1Gy) vs conditioned medium (SW1353 0Gy)

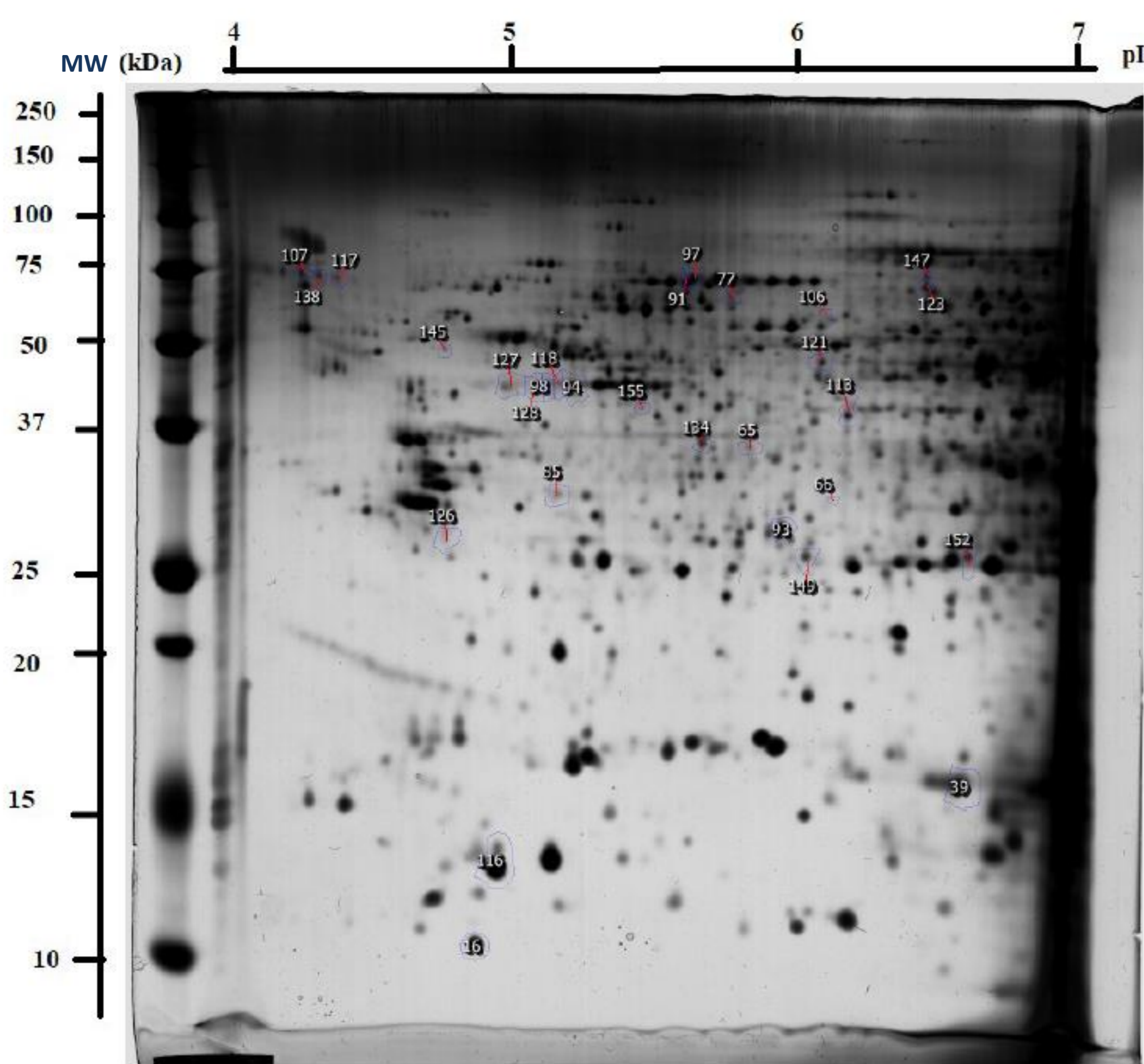
| accession | description  | Fold  |
|-----------|--|-------|
| O43790-04 | Keratin, type II cuticular Hb6                                 | 7,67  |
| P23528    | Cofilin-1  | 4,85  |
| Q13257    | Mitotic spindle assembly checkpoint protein MAD2A              | 2,83  |
| P40227-01 | T-complex protein 1 subunit zeta                               | 2,81  |
| P38159    | RNA-binding motif protein, X chromosome                        | 2,31  |
| P42167-01 | Lamina-associated polypeptide 2, isoforms beta/gamma           | 2,27  |
| Q14847    | LIM and SH3 domain protein 1                                   | 2,11  |
| P61163    | Alpha-centractin   | 2,00  |
| Q00325-01 | Phosphate carrier protein, mitochondrial                       | 2,00  |
| A6NDG6    | Glycerol-3-phosphate phosphatase                               | 1,88  |
| O00232-01 | 26S proteasome non-ATPase regulatory subunit 12                | 1,80  |
| P30566-01 | Adenylosuccinate lyase   | 1,70  |
| P30405    | Peptidyl-prolyl cis-trans isomerase F, mitochondrial           | 1,58  |
| Q99798-01 | Aconitate hydratase, mitochondrial                             | 1,54  |
| P61769-01 | Beta-2-microglobulin   | 1,50  |
| Q9NZN4-01 | EH domain-containing protein 2                                 | 1,50  |
| P54577-01 | Tyrosine-tRNA ligase, cytoplasmic                              | -1,64 |
| Q9UBM7-01 | 7-dehydrocholesterol reductase                                 | -1,67 |
| Q14152-03 | Eukaryotic translation initiation factor 3 subunit A           | -1,80 |
| P13861    | cAMP-dependent protein kinase type II-alpha regulatory subunit | -2,00 |
| P49189-01 | 4-trimethylaminobutylaldehyde dehydrogenase                    | -2,67 |

Protein extraction and separation  
 Proteins were TCA precipitated and then solubilized in laemmli buffer, and separated by SDS-PAGE.  
 Mass spectrometry  
 Each bands from the SDS-PAGE gels were picked and analysed by LC-MS/MS (Q-EX) mass spectrometry

- TNFR1 exosome secretion
- Actin regulation, ion binding and carrying
- Mitochondrial permeability
- glucose metabolism
- glucose metabolism
- Mitochondrial permeability
- MAPK pathway regulation
- Mitochondrial permeability

### Modulated proteins in bystander cells : potential bystander responses

Bystander T/C28-A2 (0,1Gy) vs control T/C28-A2 (0Gy)



Protein extraction and solubilisation  
 Proteins were extracted from TC-28/AC cells in sample buffer containing 7 M urea, 2M thiourea, 4% CHAPS, 0.05% Triton X100, 65 mM DTT, 40mM spermine, protease and phosphatase inhibitor cocktails. Proteins were then precipitated using the 2D clean-up kit (GE Healthcare) and the pellet was solubilized with TUC solution (7M urea, 2M thiourea, 4% CHAPS) and quantified with the 2D quant kit (GE Healthcare).

Protein load : 250 µg and 2D electrophoresis (6 technical / biological replicates)  
 IPG strips : pI 4-7 ; 1<sup>st</sup> dimension : Protean IEF Cell (Bio-Rad) ; 2<sup>nd</sup> dimension : 12% acrylamide gel (dodeca Cell electrophoresis unit, Bio-Rad)  
 Gel staining and picture acquisition : Silver stain, stained gels scanned on a GS 800 densitometer (Bio-Rad)

2D gel analysis and Mass spectrometry  
 Stained gels were analysed with the Progenesis SameSpots software and variant spots were picked and analysed by LC-MS/MS (Q-EX) mass spectrometry

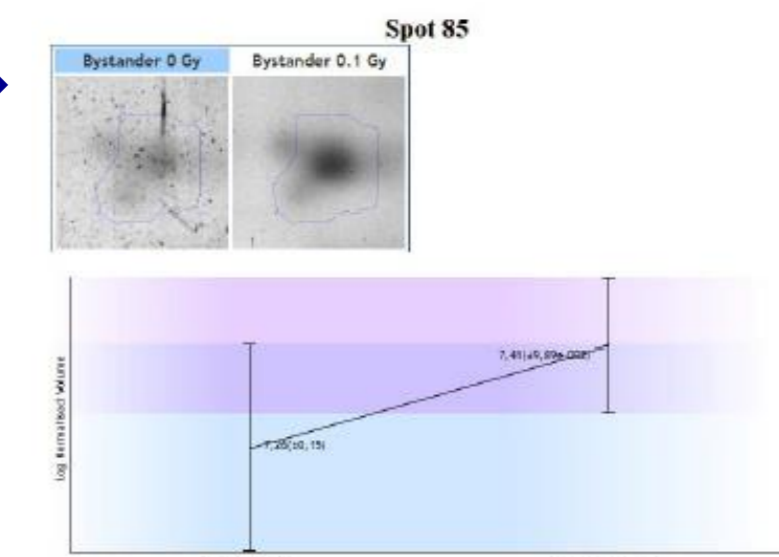
| Notes | Fold      | pI   | MW  | Accession | Description   |
|-------|-----------|------|-----|-----------|---|
| 16    | 1,3079416 | 4,79 | 20  | P60709    | Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1 - [ACTB_HUMAN]                                     |
| 39    | 1,6234021 | 6,69 | 26  | P62397    | Peptidyl-prolyl cis-trans isomerase A OS=Homo sapiens GN=PP1A PE=1 SV=2 - [PP1A_HUMAN]                    |
| 65    | 1,8130469 | 5,86 | 72  | P06793    | Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2 - [ENO1_HUMAN]  |
| 66    | 1,4032771 | 6,19 | 62  | P01383    | RIS acidic ribosomal protein P0 OS=Homo sapiens GN=RPLP0 PE=1 SV=1 - [RPL0_HUMAN]                         |
| 68    | 1,4032771 | 6,19 | 62  | x         |   |
| 77    | 1,5675063 | 6,79 | 130 | P01874    | Ig heavy chain constant alpha 1 OS=Homo sapiens GN=IGHA1 PE=1 SV=2 - [IGHA1_HUMAN]                        |
| 85    | 1,3800701 | 5,11 | 63  | Q13569    | Microtubule-associated protein RP1EB family member 1 OS=Homo sapiens GN=MAPRE1 PE=1 SV=3 - [MAPRE1_HUMAN] |
| 91    | 1,2932961 | 5,63 | 140 | P11154    | Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1 - [HSPA8_HUMAN]                      |
| 93    | 1,3063549 | 5,99 | 67  | Q06323    | Proteasome activator complex subunit 1 OS=Homo sapiens GN=PSME1 PE=1 SV=1 - [PSME1_HUMAN]                 |
| 94    | 1,3768728 | 5,18 | 88  | x         |   |
| 97    | 1,3219603 | 5,64 | 141 | P38646    | Sizes-70 protein, mitochondrial OS=Homo sapiens GN=HSPA9 PE=1 SV=2 - [HSPA9_HUMAN]                        |
| 98    | 1,5893781 | 5,09 | 88  | x         |   |
| 106   | 1,3058829 | 6,18 | 115 | x         |   |
| 107   | 1,6085417 | 4,11 | 145 | x         |   |
| 113   | 1,273887  | 6,26 | 79  | P40123    | Macrophage capping protein OS=Homo sapiens GN=CAPO PE=1 SV=2 - [CAPO_HUMAN]                               |
| 116   | 1,2721674 | 4,87 | 23  | P15926    | Desmoplakin OS=Homo sapiens GN=DSP PE=1 SV=3 - [DSP_HUMAN]  |
| 117   | 1,3195794 | 4,26 | 143 | x         |   |
| 118   | 1,4420502 | 5,13 | 88  | x         |   |
| 121   | 1,4716126 | 6,16 | 94  | P39998    | 26S proteasome regulatory subunit 7 OS=Homo sapiens GN=PSMC2 PE=1 SV=3 - [PSMC2_HUMAN]                    |
| 123   | 1,3127831 | 6,56 | 136 | P49165    | T-complex protein 1 subunit gamma OS=Homo sapiens GN=CCT3 PE=1 SV=4 - [CCT3_HUMAN]                        |
| 126   | 1,6276638 | 4,67 | 56  | P09493    | Tropomyosin alpha-1 chain OS=Homo sapiens GN=TPM1 PE=1 SV=2 - [TPM1_HUMAN]                                |
| 127   | 1,3079507 | 4,91 | 87  | P60709    | Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1 - [ACTB_HUMAN]                                     |
| 128   | 1,3653406 | 5,03 | 87  | Q13144    | Proteasomal ubiquitin receptor ADRM1 OS=Homo sapiens GN=ADRM1 PE=1 SV=2 - [ADRM1_HUMAN]                   |
| 134   | 1,2603368 | 5,68 | 73  | Q13341    | Proteasomal ubiquitin receptor ADRM1 OS=Homo sapiens GN=ADRM1 PE=1 SV=2 - [ADRM1_HUMAN]                   |
| 138   | 1,9817232 | 4,18 | 145 | P04264    | Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT11 PE=1 SV=6 - [KRT11_HUMAN]                        |
| 145   | 1,4712862 | 4,67 | 98  | Q06777    | Protein HGH1 homolog OS=Homo sapiens GN=HGH1 PE=1 SV=1 - [HGH1_HUMAN]                                     |
| 147   | 1,4495543 | 5,56 | 144 | x         |   |
| 149   | 1,3028428 | 6,09 | 91  | x         |   |
| 152   | 1,3145383 | 6,72 | 61  | x         |   |
| 155   | 1,3942451 | 5,43 | 82  | x         |   |

green spot : increased in bystander ; orange spot : decreased in bystander

→ Modulate protein folding and traffic within the cell, can be secreted, and is involved in several human pathologies

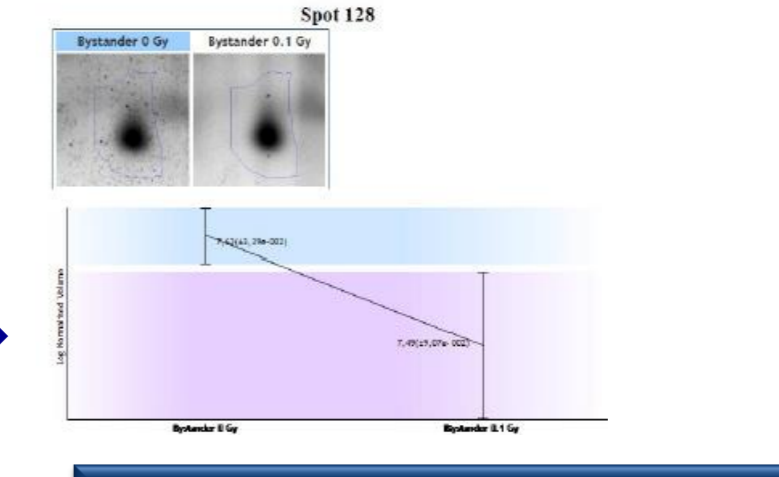
→ Enzyme with several functions, including control growth and hypoxia (interact with MBP-1)

→ Can be at the membrane or secreted



→ Oxidative stress response

→ Role in cancer cells proliferation



#### Conclusion

Several protein were observed increased or decreased and could be related to a bystander response in cells receiving conditioned medium from irradiated cells. These preliminary results need now to be validated with functional assays and the factors will be tested on other cells.