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Proteomic analysis of the Radiation-Induced Bystander Effects

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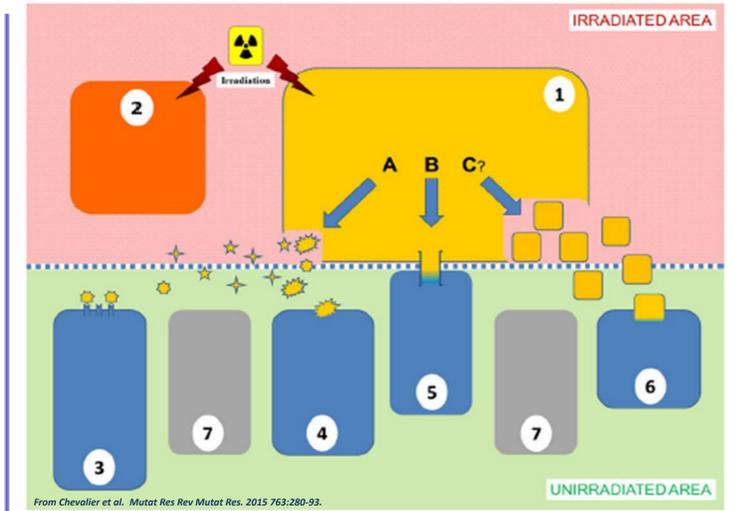


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Experimental evidence shows that, in addition to this direct and targeted effect of radiation, a side or secondary effect is observed within the un-irradiated area. Irradiated cells (1 and 2) relay a stress signal in the close proximity. Neighboring 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.

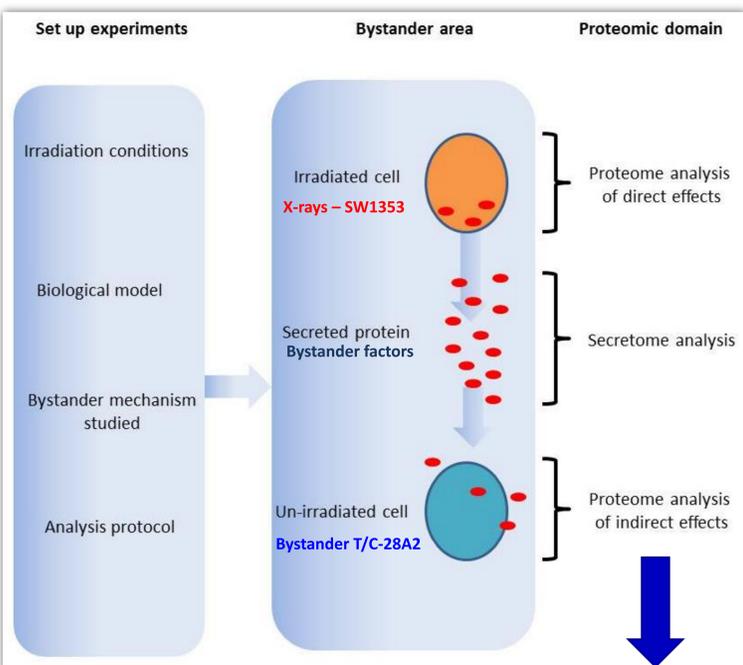
In the present study, we investigated the capacity of chondrocytes (T/C28-A2) in responding to bystander factors released by irradiated chondrosarcoma cells (SW1353) using a medium transfer protocol.



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)

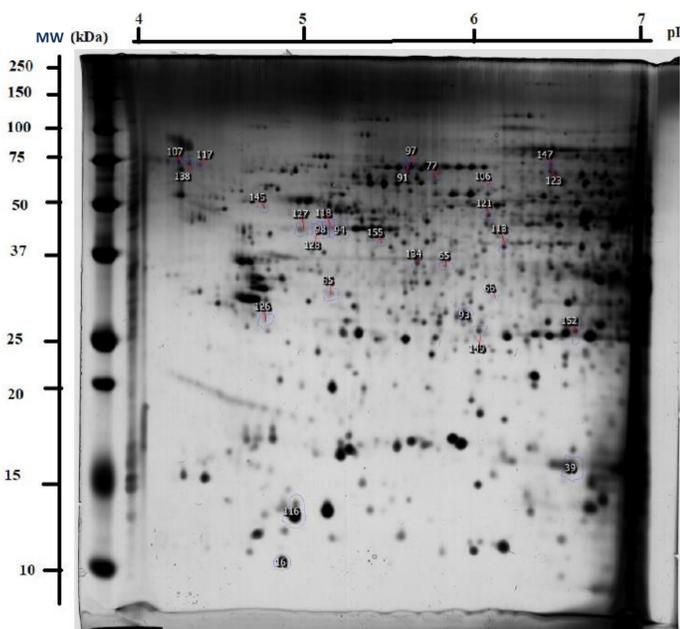
accession	description	Fold
O43790-04	Keratin, type II cuticular Hb6	7,67
P23528	Cofilin-1	4,85
Q13257	Mitotic spindle assembly checkpoint protein MAD2A	2,83
P40227-01	T-complex protein 1 subunit zeta	2,81
P38159	RNA-binding motif protein, X chromosome	2,31
P42167-01	Lamina-associated polypeptide 2, isoforms 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)



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 - [HSP70_HUMAN]
93	1,3063549	5,99	57	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	1,3056829	6,18	115	x	
107	1,6085417	4,11	145	x	
113	1,273887	6,25	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	4,25	143	x	
118	1,4420502	5,13	88	x	
121	1,4716126	6,15	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 - [CTPG_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	Eukaryotic translation initiation factor 3 subunit I OS=Homo sapiens GN=EIF3 PE=1 SV=1 - [EIF3_HUMAN]
145	1,4712862	4,67	98	Q06717	Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT11 PE=1 SV=6 - [KRT11_HUMAN]
147	1,4495543	5,56	144	x	
149	1,3028428	6,09	51	x	
152	1,3145383	6,72	61	x	
155	1,3942451	5,43	82	x	

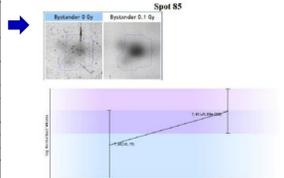
Protein extraction and solubilisation
Proteins were extracted from TC-28/AC cells in sample buffer containing 7 M urea, 2M thiourea, 4% CHAPS, 0.05% Triton X100, 65 mM DTT, 40mM spermine, protease and phosphatase inhibitor cocktails. Proteins were then precipitated using the 2D clean-up kit (GE Healthcare) 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 ; 1st dimension : Protean IEF Cell (Bio-Rad) ; 2nd 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

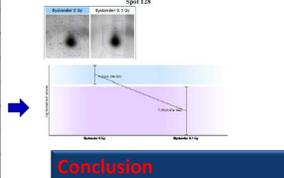
- Modulate protein folding and traffic within the cell, can be secreted, and is involved in several human pathologies
- Enzyme with several functions, including control growth and hypoxia (interact with MBP-1)

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.