Supplementary Materials

Radiation and Stemness Phenotype May Influence Individual Breast Cancer Outcomes: The Crucial Role of MMPs and Microenvironment

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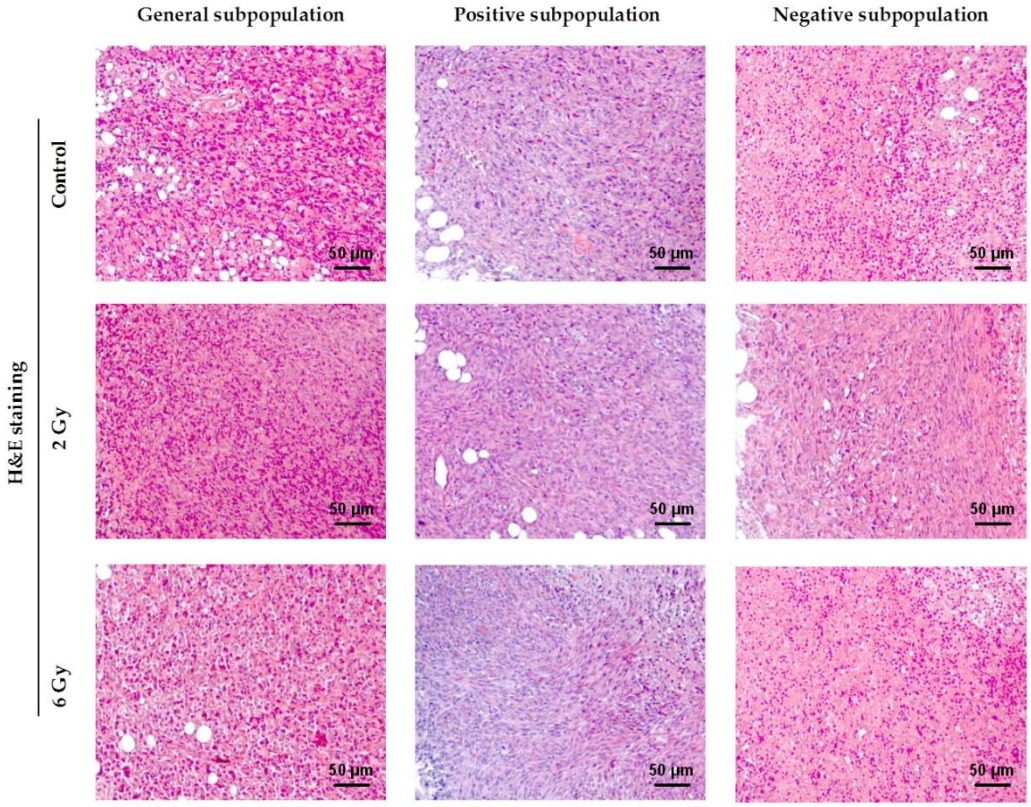

Figure S1. H&E staining of the tumors for all IR doses (0, 2 and 6 Gy) in the general, positive and negative cell subpopulations of the MDA-MB-231 cell line.

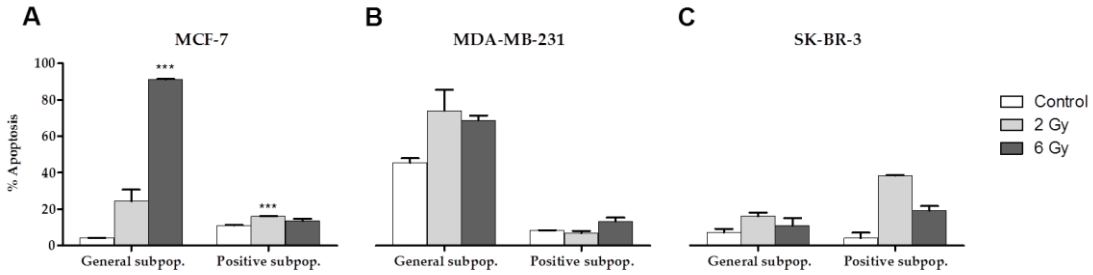

Figure S2. Values of apoptosis (%) at 0, 2 and 6 Gy IR doses measured 24 h after treatment in MCF-7 (**A**), MDA-MB-231 (**B**) and SK-BR-3 (**C**) cell lines in the general subpopulation and positive subpopulation cultures. Values are expressed as the mean ± SEM (error bars) of three independent experiments. Mean value for each experiment was calculated by averaging the triplicates ($n = 3$); *** $p < 0.001$.

Table S1. Expression values (fold change) of the genes detected in the 3D culture for all IR doses (0, 2 and 6 Gy) in the general, positive and negative cell subpopulations of the MCF-7, MDA-MB-231 and SK-BR-3 cell lines.

Genes	IR Dose	Expression Values (Fold Change)		
		General Subpopulation	Positive Subpopulation	Negative Subpopulation
MCF-7				
	Control	1.000	1.000	1.000
TIMP-1	2 Gy	1.378	0.719	0.961
	6 Gy	0.793	0.810	1.129
MDA-MB-231				
	Control	1.000	1.000	1.000
MMP-1	2 Gy	0.819	1.804	2.197
	6 Gy	0.898	2.224	1.146
	Control	1.000	1.000	1.000
MMP-3	2 Gy	1.075	1.130	0.646
	6 Gy	1.567	1.202	0.556
	Control	1.000	1.000	1.000
MMP-9	2 Gy	0.570	2.377	0.774
	6 Gy	0.655	1.584	0.675
	Control	1.000	1.000	1.000
MMP-13	2 Gy	0.413	1.286	1.563
	6 Gy	1.326	1.960	1.991
	Control	1.000	1.000	1.000
HDAC-1	2 Gy	0.740	1.234	0.441
	6 Gy	1.006	0.751	0.489
	Control	1.000	1.000	1.000
HDAC-2	2 Gy	0.980	1.256	3.150
	6 Gy	1.104	0.772	2.824
	Control	1.000	1.000	1.000
HDAC-4	2 Gy	0.900	1.131	1.266
	6 Gy	1.219	0.950	1.089
	Control	1.000	1.000	1.000
TIMP-1	2 Gy	0.777	1.048	1.290
	6 Gy	1.090	0.991	1.400
	Control	1.000	1.000	1.000
TIMP-2	2 Gy	0.837	1.254	1.230
	6 Gy	1.250	1.280	1.104
SK-BR-3				
	Control	1.000	1.000	1.000
MMP-1	2 Gy	0.384	0.822	1.160
	6 Gy	0.764	0.538	2.398
	Control	1.000	1.000	1.000
MMP-13	2 Gy	1.521	1.450	1.443
	6 Gy	1.537	1.367	1.351
	Control	1.000	1.000	1.000
HDAC-1	2 Gy	0.578	2.944	1.097
	6 Gy	0.838	0.988	4.041
	Control	1.000	1.000	1.000
HDAC-2	2 Gy	0.342	0.869	2.051
	6 Gy	0.754	0.843	1.330
	Control	1.000	1.000	1.000
HDAC-4	2 Gy	0.730	1.003	1.221
	6 Gy	1.035	0.792	0.924
	Control	1.000	1.000	1.000
TIMP-1	2 Gy	1.030	1.017	1.141
	6 Gy	1.480	0.954	1.272
	Control	1.000	1.000	1.000
TIMP-2	2 Gy	0.235	0.927	1.692
	6 Gy	0.238	1.358	1.271

Table S2. Expression values (fold change) of the genes detected in the 3D + lrECM culture for all IR doses (0, 2 and 6 Gy) in the general, positive and negative cell subpopulations of the MCF-7, MDA-MB-231 and SK-BR-3 cell lines.

Genes	IR Dose	Expression Values (Fold Change)		
		General Subpopulation	Positive Subpopulation	Negative Subpopulation
MCF-7				
	Control	1.000	1.000	1.000
TIMP-1	2 Gy	2.331	0.468	1.006
	6 Gy	0.886	1.432	1.167
MDA-MB-231				
	Control	1.000	1.000	1.000
MMP-1	2 Gy	1.276	0.771	0.224
	6 Gy	1.346	3.385	0.455
	Control	1.000	1.000	1.000
MMP-3	2 Gy	0.485	0.653	0.148
	6 Gy	0.482	1.906	0.326
	Control	1.000	1.000	1.000
MMP-13	2 Gy	1.058	1.383	0.631
	6 Gy	0.632	1.149	3.123
	Control	1.000	1.000	1.000
HDAC-2	2 Gy	8.690	0.881	0.845
	6 Gy	4.539	1.265	1.007
	Control	1.000	1.000	1.000
HDAC-4	2 Gy	0.903	1.171	1.311
	6 Gy	0.733	1.052	2.307
	Control	1.000	1.000	1.000
TIMP-1	2 Gy	1.015	1.027	1.478
	6 Gy	0.818	0.933	1.850
	Control	1.000	1.000	1.000
TIMP-2	2 Gy	0.448	1.009	1.066
	6 Gy	0.615	0.777	1.446
SK-BR-3				
	Control	1.000	1.000	1.000
MMP-13	2 Gy	0.908	0.799	1.257
	6 Gy	0.523	0.720	1.446
	Control	1.000	1.000	1.000
HDAC-2	2 Gy	0.961	0.567	0.852
	6 Gy	0.722	1.005	2.920
	Control	1.000	1.000	1.000
HDAC-4	2 Gy	1.120	0.399	0.558
	6 Gy	0.598	0.640	0.840
	Control	1.000	1.000	1.000
TIMP-1	2 Gy	1.121	1.421	2.329
	6 Gy	1.034	0.984	2.203
	Control	1.000	1.000	1.000
TIMP-2	2 Gy	1.111	0.208	0.772
	6 Gy	0.823	0.264	0.680

Table S3. Primer sequences used for the measurements of gene expression in qRT-PCR assays.

Genes		Primer Sequences
18S	Forward	CGGCGACGACCCATTCGAAC
	Reverse	GAATCGAACCCTGATTCCCCG
GADPH	Forward	CACCAGGGCTGCTTTTAACTC
	Reverse	CCTTGACGGTGCCATGGAATT
MMP-1	Forward	AAGGCCAGTATGCACAGCTT
	Reverse	TGCTTGACCCTCAGAGACCT
MMP-2	Forward	TTTCCATTCCGCTTCCAGGGC
	Reverse	TCGCACACCACATCTTTCCGT
MMP-3	Forward	GGCTTTCCCAAGCAAATAGC
	Reverse	GTGCCCATATTGTGCCTTCT
MMP-9	Forward	CCTGCCAGTTTCCATTCATC
	Reverse	GCCATTCACGTCGTCCTTAT
MMP-13	Forward	AACATCCAAAAACGCCAGAC
	Reverse	GGAAGTTCTGGCCAAAATGA
TIMP-1	Forward	ACCAGACCACCTTATACCA
	Reverse	CATTCCTCACAGCCAACA
TIMP-2	Forward	TGCTTCTTGCCTGTTCTG
	Reverse	CTGGACTTCTTACTATGCTTA
HDAC-1	Forward	AAGACGACCCTGACAAGC
	Reverse	AGACCTGGCACCCTTTAT
HDAC-2	Forward	GGTAACTCCTGCTACTAAGAT
	Reverse	GGTGCTATTGTGAATGTCTG
HDAC-4	Forward	ACCTACCTGATGCCTGTT
	Reverse	AACCTCCAACTCCACTGA

Table S4. Values of tumor volume (mm^3) corresponding to monitoring of tumor growth after orthotopic inoculation in Matrigel of the general, positive and negative cell subpopulations of the MDA-MB-231 cell line at 0, 2 and 6 Gy.

Days	IR Dose	Tumor Volume (mm^3)		
		General Subpopulation	Positive Subpopulation	Negative Subpopulation
7	Control	0.000	0.000	0.000
	2 Gy	0.000	0.000	0.000
	6 Gy	0.000	0.000000	0.000
14	Control	0.000	0.000	0.000
	2 Gy	2.339	0.936	0.000
	6 Gy	0.000	1.872	0.000
21	Control	0.000	2.495	0.000
	2 Gy	7.798	1.872	0.000
	6 Gy	0.000	2.495	0.000
28	Control	7.486	6.238	8.110
	2 Gy	10.92	2.495	3.119
	6 Gy	0.000	4.387	1.248
35	Control	7.486	6.238	8.110
	2 Gy	10.92	5.010	6.238
	6 Gy	2.495	8.842	2.495
42	Control	7.486	6.238	8.110
	2 Gy	10.92	5.010	11.22
	6 Gy	4.991	10.89	6.238
49	Control	9.982	9.982	8.110
	2 Gy	10.92	5.010	12.48
	6 Gy	4.991	11.02	7.486

56	Control	9.982	9.982	11.85
	2 Gy	10.92	7.468	14.41
	6 Gy	4.991	17.70	10.00
63	Control	9.982	10.63	13.76
	2 Gy	14.14	11.49	20.20
	6 Gy	4.991	20.73	14.16
70	Control	13.76	10.92	15.05
	2 Gy	18.96	16.36	20.20
	6 Gy	4.99	30.36	19.32
77	Control	15.05	14.11	22.77
	2 Gy	20.57	35.05	45.88
	6 Gy	4.991	30.36	32.080
84	Control	17.63	22.86	33.44
	2 Gy	58.77	35.95	58.92
	6 Gy	4.991	31.33	75.79
91	Control	20.81	40.88	57.40
	2 Gy	287.3	43.89	104.2
	6 Gy	7.486	44.308	93.70
98	Control	69.46	76.85	63.91
	2 Gy	313.3	208.0	118.0
	6 Gy	19.44	97.76	96.27
105	Control	110.1	90.14	118.3
	2 Gy	348.7	208.0	126.6
	6 Gy	72.96	106.9	96.27
112	Control	245.66	146.9	174.0
	2 Gy	368.96	256.1	149.8
	6 Gy	126.1	209.5	110.9
119	Control	408.1	268.4	217.5
	2 Gy	413.2	279.0	194.4
	6 Gy	314.0	221.3	159.2

Table S5. *p* values of tumor volume when comparing the doses of 2 and 6 Gy with the control within each cell subpopulation.

Days	***p* Values**					
	General Subpopulation		**Positive Subpopulation**		**Negative Subpopulation**	
	2 Gy vs. Control	**6 Gy vs. Control**	**2 Gy vs. Control**	**6 Gy vs. Control**	**2 Gy vs. Control**	**6 Gy vs. Control**
7	1.000	1.000	1.000	1.000	1.000	1.000
14	0.180	1.000	0.317	0.317	1.000	1.000
21	0.059	1.000	0.655	1.000	1.000	1.000
28	0.414	0.063	0.180	0.715	0.038	0.042
35	0.414	0.194	0.655	0.500	0.180	0.066
42	0.414	0.581	0.655	0.593	0.180	0.180
49	0.655	0.317	0.141	1.000	0.102	0.317
56	0.655	0.317	0.500	0.593	0.109	0.180
63	0.141	0.317	0.892	1.000	0.102	0.317
70	0.276	0.102	0.892	1.000	0.102	0.593
77	0.269	0.102	0.892	0.686	0.144	0.686
84	0.144	0.102	1.000	0.500	0.225	0.225
91	0.068	0.068	0.715	0.500	0.225	0.225
98	0.144	0.138	0.080	0.500	0.225	0.345
105	0.144	0.500	0.080	0.500	0.893	0.686
112	0.465	0.043	0.043	0.500	0.686	0.225
119	0.715	0.080	0.893	0.500	0.686	0.225

Table S6. *p* values of tumor volume when comparing the positive and negative subpopulations with the general subpopulations.

Days	IR Dose	*p* Values	
		Positive Subpop. vs. General Subpop.	**Negative Subpop. vs. General Subpop.**
7	Control	1.000	1.000
	2 Gy	1.000	1.000
	6 Gy	1.000	1.000
14	Control	1.000	1.000
	2 Gy	0.383	0.094
	6 Gy	0.317	1.000
21	Control	0.136	1.000
	2 Gy	0.068	0.006
	6 Gy	0.136	1.000
28	Control	0.746	0.911
	2 Gy	0.022	0.022
	6 Gy	0.136	0.134
35	Control	0.746	0.911
	2 Gy	0.205	0.223
	6 Gy	0.190	1.000
42	Control	0.746	0.911
	2 Gy	0.205	0.866
	6 Gy	0.265	0.729
49	Control	0.204	0.408
	2 Gy	0.205	0.264
	6 Gy	0.265	0.502
56	Control	0.204	0.881
	2 Gy	0.258	0.109
	6 Gy	0.156	0.326
63	Control	0.435	0.180
	2 Gy	0.451	0.203
	6 Gy	0.156	0.042
70	Control	0.063	0.513
	2 Gy	0.211	1.000
	6 Gy	0.156	0.032
77	Control	0.331	0.045
	2 Gy	0.133	0.213
	6 Gy	0.156	0.022
84	Control	0.827	0.042
	2 Gy	0.135	1.000
	6 Gy	0.108	0.022
91	Control	1.000	0.073
	2 Gy	0.025	0.086
	6 Gy	0.280	0.008
98	Control	0.196	0.916
	2 Gy	0.327	0.086
	6 Gy	0.169	0.026
105	Control	0.341	0.751
	2 Gy	0.221	0.086
	6 Gy	0.754	0.463
112	Control	0.175	0.347
	2 Gy	0.221	0.027
	6 Gy	0.347	0.917
119	Control	0.347	0.175
	2 Gy	0.221	0.014
	6 Gy	0.754	0.347

Table S7. Values of MMP-1 positive staining (% tumor area) corresponding to IHC staining of the tumors from the different conditions studied (IR doses and cell subpopulations).

Subpopulation	MMP-1 Positive Staining (% Tumor Area)		
	Control	2 Gy	6 Gy
General	42.42	43.30	36.66
Positive	34.09	35.01	30.78
Negative	30.04	28.36	24.26

Table S8. Values of apoptosis (%) of the MCF-7 (A), MDA-MB-231 (B) and SK-BR-3 (C) cell lines in the general subpopulation and positive subpopulation cultures at 0, 2 and 6 Gy IR doses.

IR Dose	Apoptosis Values (%)	
	General Subpopulation	Positive Subpopulation
MCF-7		
Control	4.250	11.15
2 Gy	24.40	16.16
6 Gy	91.25	13.65
MDA-MB-231		
Control	45.50	8.400
2 Gy	74.05	6.950
6 Gy	68.85	13.40
SK-BR-3		
Control	7.150	4.150
2 Gy	16.10	38.45
6 Gy	11.15	19.25