

Genetic upregulation of activated protein C mitigates delayed effects of acute radiation exposure in the mouse plasma

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LC gradient and MS instrument parameters

Untargeted metabolomics

For untargeted metabolomics data acquisition, each sample (1 μ L) was injected to a 1.7 μ m, 2.1 mm $\times$ 50 mm Acquity BEH C18 column (Waters Corporation, MA, USA) using an Acquity UPLC system connected to an electrospray ion source coupled with a quadrupole time-of-flight mass spectrometer (ESI-Q-TOF, Xevo-G2S, Waters Corporation, MA, USA) operating in positive and negative ionization mode. The gradient mobile phases consisted of 100% water with 0.1% formic acid (solvent A), 100% acetonitrile with 0.1% formic acid (solvent B), 100% isopropanol with 0.1% formic acid (solvent C). Each sample injection was run for 13 minutes at a flow rate for 500 μ L/min. The LC gradient conditions with a ramp curve of 6 at each step were as follows: Initial – 98% A, 2% B till 0.5 minutes; 4.0 minutes – 40% A, 60% B; 8.0 minutes – 2% A, 98% B till 9.0 minutes; 9.5 minutes – 11.8% B, 88.2% C till 11 minutes; 11.5 minutes – 50% A, 50% B, 12.0 minutes – 98% A, 2% B till 13 minutes. Positive mode had a capillary voltage of 2.75 kV, a sampling cone voltage of 30 V, and a source offset of 80 V. Negative mode had a capillary voltage of 2.75 kV, a sampling cone voltage of 20 V, and a source offset of 80 V. The desolvation gas flow was 600 L/h. and the temperature was set to 500 °C. The cone gas flow was 25 L/h, and the source temperature was 100 °C. The data were acquired in the sensitivity MS Mode with a scan time of 1.000 seconds, and inter-scan delay at 0.014 seconds. Real-time mass correction was applied using a solution of Leucine-Enkephalin (1.0 ng/ml) [M+H]⁺ (m/z 556.2771), [M–H][–] (m/z 554.2615) in 500 ml of a mixture containing acetonitrile: water (50:50) and 250 μ L formic acid at an infusion rate of 20 μ L/min utilizing the Waters Lockspray® interface. Before and after samples run, a mixture of six standards (acetaminophen: m/z 152.0712 [M+H]⁺/150.0555 [M–H][–], sulfaguanidine: m/z 215.0603 [M+H]⁺/213.0446 [M–H][–], sulfadimethoxine: m/z 311.0814 [M+H]⁺/309.0658 [M–H][–], Val-Tyr-Val: m/z 380.2185 [M+H]⁺/378.2029 [M–H][–], terfenadine: m/z 472.3216 [M+H]⁺ and leucine-enkephalin: m/z 556.2771 [M+H]⁺/554.2615 [M–H][–]) were run to ensure mass accuracy during data acquisition (Supplementary Figure 3 and 4).

Targeted metabolomics

A binary solvent comprising of water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B) was used. The extracted metabolites were resolved at 0.2 mL/min flow rate starting with 100% of solvent A and hold for 2.1 minutes and moving to 5% of solvent A over a time period of 12 minutes and hold for 1 minute and equilibrating to initial conditions over a time period of 7 minutes using auto sampler temperature 15 °C and oven temperature 30 °C. Source and gas setting for the method were as follow: curtain gas = 35, CAD gas = medium, ion spray voltage = 2500 V in positive mode and –4500 V in negative mode, temperature = 400 °C, nebulizing gas = 60 and heater gas = 70.

Targeted Lipidomics

A binary solvent comprising of acetonitrile/water 95/5 with 10 mM ammonium acetate as solvent A and acetonitrile/water 50/50 with 10 mM ammonium acetate as solvent B was used for the resolution. Lipids were resolved at 0.7 mL/min flow rate, initial gradient conditions started with 100% of solvent A, shifting towards 99.9% of solvent A over a duration of 3 minutes, 94% of solvent A over a time period of 3 minutes and 25% of solvent A over a period of 4 minutes. Finally, washing with 100% of B for 6 minutes and equilibrating to initial conditions (100% of solvent A) over a time period of 6 minutes using auto sampler

temperature 15 °C and oven temperature 35 °C. Source and gas setting were as follow: curtain gas = 30, CAD gas = medium, ion spray voltage = 5.5 kV in positive mode and -4.5 kV in negative mode, temperature = 550 °C, nebulizing gas = 50 and heater gas = 60.

[illegible]

[illegible]

Supplementary Table 3. List of dysregulated metabolites and lipids upon exposure to 2.5 Gy of gamma radiation, 6 months' post irradiation

Med	Protein	APC11										APC10																									
		F0					F1					F0					F1																				
		Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein																
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