GATA3 silencing in human keratinocytes leads to global transcriptional deregulation after low-dose irradiation

F. Bonin[a], M. Molina[a], C. Malet[b], C. Ginestet[b] and J. Lamartine[a]

[a] *CNRS UMR5534 & Université Claude Bernard Lyon I, Centre de génétique Moléculaire et Cellulaire, 69622 Villeurbanne, France;* [b] *Centre Léon Bérard, Service de Radiothérapie, 69008 Lyon, France*

jerome.lamartine@univ-lyon1.fr

Skin is the most exposed organ to various environmental aggressors, including ionizing radiations. Low-dose exposure to ionizing radiations account for most occupational, medical and environmental irradiations. In our group, we are studying the effects of low-dose exposure on epidermal keratinocytes, the main target of radiations in the outermost layer of the skin. We have previously shown (Franco, Lamartine et al., Radiation Research 2005) that low-dose exposure to gamma rays induces specific gene regulations in normal human keratinocytes. By a bioinformatic approach, we have studied the promoter sequences of genes sharing molecular signatures to γ-rays and found common binding sites for transcription factors including GATA3, a protein known to play a role in keratinocytes renewal and differentiation. The binding of GATA3 on these promoter sequences was confirmed by chromatin immunoprecipitation. To go further into the role of GATA3 after exposure to ionizing radiations, we studied the cellular and molecular consequences of radiations in cells where GATA3 was knocked-down. This silencing was obtained by lentiviral-mediated expression of a shRNA targeting the GATA3 transcript in differentiated keratinocytes. We first evaluated the radiosensitivity of these cells : we didn't observe any additional toxicity of 2 Gy and 1 cGy radiations in GATA3 silenced cells in term of immediate survival (addressed by XTT test) and long term colony forming efficiency. We also investigated the consequences of the GATA3 silencing on the transcriptome of X-rays irradiated cells. Oligonucleotide microarrays were used to assess transcriptional changes over a time-course between 3 and 72h post-irradiation in a GATA3 knocked-down background or in a normal background. We observed a completely modified transcriptomic response in GATA3 silenced cells with a strong peak of induction/repression 48h after irradiation. Functional annotation of these genes revealed a potential involvement of the p38 MAPK signaling pathway, which is known to be activated after various environmental stresses and to modulate cell proliferation. We are now pursuing additional experiments to validate this hypothesis, and to understand the link between GATA3 and the deregulated genes. Altogether, these results reveal that GATA3 plays a complex role in the response of human keratinocytes to very low doses of irradiation by regulating a specific set of genes.

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