The influence of the keratin cytoskeleton on the cell response caused by ionizing radiation and death receptor cytokines

Epidermolysis bullosa simplex (EBS) is a keratin-linked skin fragility disorder, caused by the presence of a severe keratin 5 or 14 gene mutation. It has been shown that these mutations place EBS keratinocytes in a state of stress and lead to a continuous activation of JNK, ERK and p38 signaling pathways. Also, skin tissue fragility in the severe EBS Dowling-Meara (EBS-DM) phenotype appears to be the combined result of loss of keratin cytoskeleton function, and the consequently decreased expression of many cell junction and cell adhesion proteins. Recently, it has been reported that EBS-DM patients have an increased cumulative risk for basal cell carcinoma after the age of 40. Using protein microarrays we tested 500 proteins with a key role in different signaling pathways. Out of the resulting 42 proteins with significantly altered expression levels in the EBS-DM mutant, 1/3 of them involved components of the death receptor signaling pathway (TRAIL, DR3, DR4, DR5, DcR1, DcR2, TNFRII, etc), a number of pro-survival and pro-apoptosis components of the intrinsic apoptotic signaling pathway (BID, Bcl-XL, etc) and a number of DNA damage response proteins such as P53BP1 and PUMA. Using 3 previously described EBS-DM patient-derived and 2 control keratinocyte cell lines, cell metabolic activity and cell proliferation was measured to test the effectiveness of DNA damage response mechanisms after exposure to ionizing radiation (IR) and sensitivity to TRAIL and TNF treatment. In comparison to control cell lines, EBS-DM mutants displayed lower cell count and decreased metabolic activity followed up to 7 days after the irradiation. Also, in the clonogenic assay EBS-DM cell lines produced more clones than control cell lines. Western blot analysis of the DNA damage response pathway suggested marked differences between control and EBS-DM cell lines. Based on this data we believe that to overcome the consequences of severe keratin (5 or 14) mutations, EBS-DM keratinocytes have to adjust their metabolism. A part of this process is also a change in their DNA damage response mechanism, which fails to respond properly and under such circumstances mutant keratinocytes may also become more susceptible to malignant transformation.