Assessment of the p53 response of keratinocytes in culture and in intact human skin.

Raymond L. Warters[1], Sergey B. Zhuplatov[1], Dustin L. Williams[1] and Sancy A. Leachman[2]. [1]Department of Radiation Oncology and [2]Department of Dermatology, University of Utah Health Sciences Center, Salt Lake City, UT 84132

Purpose. The purpose of this study was to compare the regulation of the p53 stress response of cultured keratinoctyes with keratinocytes in intact human skin.

Methods. Neonatal human skin was obtained from the LDS Hospital Newborn Clinic under University of Utah IRB # 8476-00. Primary, low passage number keratinocytes were recovered from Dispase II-treated neonatal skin using standard methods and cultured in serum free keratinocyte medium (SFKM) (Invitrogen). Skin epidermis was peeled away from skin that was heated at 60°C for 60 seconds and placed into a RIPA buffer. Proteins were recovered by dissolving the skin epidermis into RIPA buffer by FastPrep lysis and sonication. Equal amounts of protein from cultured cells or the epidermis were separated through 4-12% gradient acrylamide gels, electroblotted onto membranes and western analyzed for the expression of specific protein epitopes by standard methods.

Results. Per equal μg protein loaded onto acrylamide gels, we recovered approximately equal amounts of histone proteins, vimentin and Lamin A/C protein in extracts from cultured keratinocytes, as well as in extracts of intact epidermis. These results are consistent with our extraction approach recovering primarily cell (i.e., keratinocyte) proteins from the intact skin epidermis. Per μg of protein recovered from cultured keratinocytes, we observed significant levels of the p53 and Mdm2 proteins. The relative amount of p53 per cultured keratinocyte increased as the level of confluence increased, with a 10-fold increase in p53 between 10 to 90% confluence. In contrast, we recover little or no p53 protein in the protein extracted from the intact neonatal skin. We recovered equivalent amounts of the Mdm2 protein from both lysed skin epidermis and from cultured keratinocytes.

Conclusions. 1) Our results are consistent with an efficient recovery of cell protein from the epidermis of intact human skin. 2) Our results are also consistent with there being a characteristic difference in the regulation of at least one important IR-induced stress response, i.e., the p53 DNA damage stress response, in cultured keratinocytes relative to keratinocytes in the intact human skin. Since the Mdm2 protein is an E3 ubiquitin ligase that maintains low p53 protein levels through the action of the 26S Proteasome, our results indicate that in the epidermis the levels of p53 may be kept low through Mdm2 ubiquitylation of the p53 protein. This appears not to be the mechanism of regulating p53 levels in cultured keratinocytes. This work was supported by Department of Energy grant number DE-FG03-01ER63240.