In E6E7-immortalized human keratinocytes, [HRasG12V-GTP] high levels block autophagic flux leading to cell death

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Introduction: Keratinocytes immortalized by E6E7 is the most studied model of epithelial cell oncogenesis. Ectopic expression of E6 and E7 in human keratinocytes primary cultures yields non-tumorigenic immortalized cellular sublines, which can be experimentally transformed with HRas^{G12V}, mainly working cooperatively with cMyc, two of most found oncoprotein mutated in cervical cancer.

It is largely accepted that these keratinocyte culture phenomena closely mimic the development of cancer in stratified squamous epithelium. However, the importance of HRas^{G12V} and cMyc in the cell cycle control and the functional interactions between E6/E7 and those proteins remain essentially unknown or ambiguous.

Objective: To analyze the effect of high expression of HRas^{G12V} in E6/E7-immortalized keratinocytes. We hypothesize that the outcome of HRas^{G12V} expression in an E6E7-keratinocyte background depends on [HRas^{G12V}-GTP] levels, leading the cells to different fates.

Rationale and Methods: We infected E6E7-keratinocytes with a ER:HRas^{G12V}-carrying viral vector, yielding E6E7-ER:HRas^{G12V}-stable sublines. 4OH-Tamoxifen (an estrogen analogue) promotes liberation of ER:HRas^{G12V}, allowing its activation to ER:HRas^{G12V}-GTP. Intracellular levels of [ER:HRas^{G12V}-GTP] can be experimentally controlled by the extracellular levels of [4OHT]. Variables of the E6E7-ER:HRas^{G12V}–keratinocyte sublines were analyzed and quantified: rate of proliferation; cell cycle progression and death; morphology; [ER:HRas^{G12V}-GTP].

Results and Conclusions: At high levels of [ER:HRas^{G12V}-GTP], E6E7-ER:HRas^{G12V}-keratinocytes showed cell-cycle arrest at G2/M phase, just after 48h of ER:HRas^{G12V} induction, causing proliferation inhibition and/or apoptosis. In addition, we observed huge quantities of accumulated autophagosomes, which seemed to result from a block in autophagic flux, leading to cell death. Furthermore, we observed an inverse relation between HRas^{G12V} and cMyc levels. Apparently, elevated levels of [ER:HRas^{G12V}-GTP] paradoxically led to cMyc degradation, immediately before the starting of the autophagic process. These observations suggest that oncogenic stress caused by increasing levels of [ER:HRas^{G12V}-GTP] results from a autophagy flux block due to cMyc degradation.

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