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Editorial

Triggering Invasiveness of Partially Transformed Oral Keratinocytes

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This study tests the hypothesis that invasion of partially transformed keratinocytes is initiated by diffusible, proinvasive signals provided by species-specific fibroblasts. In vitro organotypic cultures of neoplastic human oral mucosa were constructed by growing a partially transformed, nontumorigenic keratinocytic cell line isolated from a dysplastic human oral lesion (DOK-ECACC94122104) on top of various types of connective tissue equivalents. Cultured tissues were analyzed by histomorphometry (depth and area of invasion: Dinv, Ainv) and immunohistochemistry. Presence of human fibroblasts in the matrix induced a local invasion of DOK (Dinv = 95.6 ± 7.1 µm, Ainv = 45.8 ± 3.5%). Minimal invasion ($P < 0.05$) was observed when DOK grew on simple collagen matrix (Dinv = 14.1 ± 2.1 µm, Ainv = 3.7 ± 0.8%) or matrices containing fibroblasts from mouse (Dinv = 11.5 ± 4.0 µm, Ainv = 4.3 ± 1.0%) or rat (Dinv = 15.6 ± 1.2 µm, Ainv = 6.1 ± 0.5%). In these cultures, local invasion could be induced by the presence of human fibroblasts in a bottom layer of the collagen matrix ($P < 0.05$) or by conditioned medium from organotypic cultures of DOK on human fibroblast-containing matrix ($P < 0.05$) but not by conditioned medium from human fibroblast monocultures ($P > 0.05$). Deposition of human collagen IV was observed at epithelial-matrix interface only when DOK behaved invasively. In conclusion, invasion of partially transformed oral keratinocytes was triggered by keratinocyte-induced fibroblast-derived diffusible factor(s) in a species-specific manner and associated with de novo synthesis of collagen IV. An increasing number of reports suggest that fibroblasts from tumor stroma actively contribute to malignant progression of epithelial neoplasms. Both normal and activated carcinoma-associated fibroblasts have been shown to enhance in vitro invasiveness of human squamous cell carcinoma (SCC) cell lines. Several reports of experiments done on monolayer cultures have suggested that this effect could be attributable to diffusible factors synthesized by fibroblasts. However, it has been suggested recently that the results from studies performed on conventional, two-dimensional monolayer cell culture models are difficult to extrapolate to the in vivo situation because they do not account for the much more complex mechanisms involved in the three-dimensional process of cancer development and invasiveness. Thus, further experimental evidence from more complex in vitro organotypic models that closely mimic the in vivo three-dimensional tissue structure and cell-to-cell interactions is needed to identify the specific fibroblast-related factor(s) of major importance for in vivo invasiveness of SCCs. On the other hand, the previous studies have used fully transformed cell lines with an established in vivo invasive phenotype. Whether the same fibroblast-dependent mechanism(s) of invasiveness takes place at earlier stages of keratinocyte transformation, when local invasion is initiated, it is not yet known, and therefore it has been the focus of this investigation. Another fibroblast-related factor of importance for the in vivo invasiveness of carcinoma cells seems to be the origin of fibroblasts. A role for organ specificity of fibroblasts in promoting carcinoma cell invasion has been shown. Several studies have reported that only a limited number of tumour cells of human origin grew in nude mice. The sensitivity of the in vivo malignancy test for human neoplastic cells, especially those at early stages of malignancy has been questioned, and attempts to develop functionally reliable in vivo experimental models of human tissues in mice have been done by humanizing the mice microenvironment through addition of human fibroblasts before xenotransplantation of the human epithelial cells, but the issue of species specificity has not been further investigated partially because of the lack of appropriate experimental models. The later development of heterologous organotypic models has made possible such studies by construction of models harboring cell types from different species. In the present study we have used such organotypic models 1) to test the hypothesis that invasion of partially transformed oral epithelial cells is triggered by diffusible, proinvasive signal(s) provided by species-specific oral fibroblasts; 2) to investigate whether the growth factors suggested by monolayer culture studies to be important for SCC invasiveness play a key role for local invasiveness of partially transformed oral keratinocytes in organotypic three-dimensional cultures; and 3) to identify specific phenotypical changes of partially transformed oral keratinocytes associated with the transition from a noninvasive to an invasive behavior. Partially transformed human oral keratinocytes were obtained from The European Collection of Cell Cultures and routinely grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 20 µg/ml l-glutamine, and 5 µg/ml hydrocortisone. Primary normal human oral fibroblasts were isolated from six biopsies of human buccal mucosa, after surgical removal of wisdom teeth as previously described. the study, approved by the Ethics Committee of the University of Bergen, included clinically healthy donors only after informed consent. Research was performed at the Department of Odontology, Oral

Pathology and Forensic Odontology. Normal mouse oral fibroblasts were isolated from the buccal mucosa of six B6D2F mice, and normal rat oral fibroblasts were isolated from the buccal mucosa of six BD IX rats following the same procedure. Animal care was in accordance to national legislation and institutional guidelines. Primary human fibroblasts were routinely grown in minimum essential medium Eagle. Primary mouse and rat fibroblasts were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 20 µg/ml l-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 0.25 µg/ml amphotericin B. The cultured tissues were harvested at day 10 of co-culture. One half of each culture was snap-frozen in isopentane prechilled in liquid nitrogen, and the other half was fixed in 4% buffered formalin, pH 7.15, and embedded in paraffin. Experiments were run six times, in duplicates.