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Vital Cell Labelling for the Detection of Invasive Growth in the Chick Embryo Skin Invasion Assay

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Abstract Of the various *in vitro* invasion assays described, only a few use tissues as substrates, for example, the chick heart fragment assay and the chick embryo skin (CES) invasion assay (Noguchi *et al.*, 1978). We have improved culture conditions for the CES invasion assay (Schlage, 1989). A suspension of neoplastically transformed cells is incubated on an explanted piece of skin from a 9–10-day-old chick embryo. After 1, 2 and 3 days, the explants are fixed and cross-sectioned. As a measure of invasiveness, the number of invading cells and their mitotic activity should be evaluated. We tested the suitability of the vital fluorescence dye PKH-2 (Horan and Slezak, 1989) for improving discrimination between CES cells and invading cells: human cervical carcinoma (HeLa), 3-methylcholanthrene (MCA)-transformed mouse embryo fibroblasts (10T½ 2-MCA7), and MNNG-transformed rat tracheal epithelial cells (RTE-MNNG). The three cell lines formed distinct infiltrates on days 2 and 3, but on day 1, only 10T½ 2-MCA7 and RTE-MNNG cells formed infiltrates. Although PKH-2 labeling was found suitable for the detection of invasion, cellular resolution and dye stability in cryosections is still unsatisfactory. To overcome this for routine work, we suggest improving the histological processing and using PKH-26, which is a more stable dye.

Keywords: chick embryo; epidermis; *in vitro* invasion test; neoplastic transformation; PKH-2 dye.

Abbreviations: CES = chick embryo skin; FBS = foetal bovine serum; MCA = 3-methylcholanthrene; MNNG = *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine.

INTRODUCTION

Malignant tumours and their metastases consist of neoplastically transformed, "malignant" cells. To characterize the malignant potential of transformed cells, their cytogenetic alterations are a necessary but not sufficient criterion. To decide whether genetically transformed cells, for example from tumour biopsies or following chemical transformation *in vitro*, exhibit the malignant phenotype, investigations on their *in vivo* tumorigenicity or on their *in vitro* behaviour, particularly invasiveness, are required.

In vitro assays for invasiveness have been designed with a variety of test substrates, either acellular (e.g. filter membranes, gel matrices, placenta membrane) or tissue-based, for example, chick heart fragment (Mareel *et al.*, 1979) or chick embryo skin (CES) (Noguchi *et al.*, 1978). The latter models may mimic the real-life situation better than models with simpler invasion matrices. Noguchi *et al.* (1978) and others (Bather *et al.*, 1985; Donahoe *et al.*, 1982; Katoh and Charoensiri, 1989; Rutzky *et al.*, 1983) have demonstrated the strong correlation between *in vivo* neoplastic behaviour and *in vitro* invasiveness in the CES invasion assay for a variety of transformed human cell lines, using deepness of invasion and mitotic activity as assay criteria.

Although we have improved the assay design (Schlage, 1989), it remains difficult to discriminate between invading cells (particularly of rodent origin) and CES tissue on histological sections. Obviously, a specific marker is required. We therefore tested various markers to improve the identification of invading cells. As commercial antibodies specific for the variety of cell types and species were not readily available, we tested a newly developed vital fluorescent dye. This dye, PKH-2, stably integrates into the plasma membrane of cells and does not interfere with cell growth (Horan and Slezak, 1989). Our objective was to test the suitability of the PKH-2 dye as a marker for identifying invading cells in the CES invasion assay.

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