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The enigma of carcinogenesis – stroma or epithelial cells?

A principle of contemporary cancer biology is that tumours develop on account of the accumulation of genetic alterations in oncogenes and tumour-suppressor genes (Hanahan and Weinberg 2000). Many findings, principally with the rat liver model, are responsible for the widespread belief in the multi-hit, multi-step hypothesis, which holds that cancer evolves in progressive steps (Scherer 1984). The hypothesis is that a mutation occurs in a somatic cell; if the mutation is not eliminated or corrected, the mutant cell proliferates, keeps accumulating further mutations, and finally assumes a phenotype described by the term 'malignant transformation'. A plethora of papers on carcinogenesis, using experimental models as well as clinical samples, tell us that a mutated cell, when exposed to certain forms of insult from the microenvironment (reactive oxygen species, hormones, metabolites, etc.) or macroenvironment (infections, chemical or physical carcinogens, etc.), undergoes genetic instability and progresses to a cancer.

The mammary epithelium, which forms the branched alveolar glands, is closely associated with the stroma, which is made up of connective and adipose tissue. Stromal-epithelial interactions regulate tissue homeostasis and can influence tumorigenesis and metastasis (Radisky *et al* 2001). Epithelial cells in mammary terminal end buds, or terminal ductules, are thought to be the targets of carcinogenic initiation, and a series of morphologically identifiable steps are believed to accompany the development of a mammary carcinoma.

Recent findings by Maffini *et al* (2004) go against this generally accepted picture of mammary carcinogenesis. The authors argue from the results of elegant experiments that (i) it is the stroma of rat breast tissue that is the target of carcinogens such as N-nitrosomethyl urea (NMU), and (ii) mutations (such as in the Ha-ras gene, a common target for NMU) in the epithelial cells themselves have no role to play in carcinogenesis. They have carried out tissue recombination experiments using mammary gland stroma (cleared fat pad) and mammary epithelial cells grown *in vitro*. Epithelial cells, treated either with NMU or the vehicle alone (0.85 g/l NaCl solution), were transplanted into cleared fat pads, which in turn were exposed to NMU or vehicle alone. Epithelial cells treated *in vitro* with NMU did not form tumours and appeared histologically similar to normal mammary gland tissue. On the other hand, in combinations in which the stroma was treated with NMU, untreated epithelial cells were transformed into tumours similar to those seen in NMU-treated rats. Maffini *et al* (2004) also found that more than 80% of neoplasias as well as the *normal* mammary gland epithelium and stroma showed Ha-ras-1 mutations. They conclude that Ha-ras-1 mutations in the transformed tissue are neither necessary nor sufficient for neoplastic transformation. How should one react to such a conclusion, which goes against the prevailing dogma?

Previous studies on breast cancer in transgenic mice have established that mutations in *ras* as well as *myc* can lead to breast cancers. A *ras* or *myc* mutation appears to be sufficient to cause breast cancer to develop in a transgenic animal, but tumour onset is substantially enhanced when the two are overexpressed simultaneously (Hulit 2001). In the NMU-induced rat mammary carcinoma model, almost 90% of the tumours show Ha-ras mutations (Zarbl *et al* 1985). However, Cha *et al* (1996) found that although activating *ras* mutations arise spontaneously in the mammary epithelium of female Fischer 344 rats during normal development, their oncogenic potential is realized only under specific physiological conditions, for example, exposure to a carcinogenic dose of NMU. NMU promoted the outgrowth of these pre-existing mutants. Given that, it is not surprising that *ras* mutations were observed even in untreated rat mammary glands. Tumours would arise only when the *ras*-mutation carrying cells are further treated with carcinogens such as NMU.

Finally, susceptibility to carcinogenesis varies considerably between different rat strains. It is the same in humans – millions of humans may be exposed to comparable doses of tobacco-derived carcinogens, but only a small fraction of them ever get cancer. Wistar Fruth, a strain of rats used by Maffini *et al* (2004), is a highly susceptible strain, whereas the Copenhagen (Cop) rat strain is highly resistant to both spontaneous and induced mammary carcinogenesis (Wang *et al* 1991). The resistance of Cop rats to NMU is not because *ras* mutations cannot occur, but is because the mutated *ras*-carrying cells are unable to undergo sustained clonal expansion (Wood *et al* 2002). It would be interesting to see if Cop rat mammary epithelial cells form tumours when associated with the NMU-treated stroma of Wistar Fruth rats in a tissue recombination experiment. If Maffini *et al* (2004) are correct, and the stroma is the target of the carcinogens, tumours should arise in such reconstruction experiments. But one should keep in mind the possibility that tumours could arise from NMU-treated host adult stem cells (ASCs). This could also be the case in Maffini *et al*'s experiments, in which they used treated whole rats with cleared fat pads. These could still be having ASCs, which – not the stroma – could be the target for NMU. The point can be cleared up by examining the origin of the tumour cells in the reconstruction experiments – whether they arise from Wistar Fruth or from Cop rats. Until then the enigma of mammary carcinogenesis continues.

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