

# Infiltrating CD11b<sup>+</sup>CD11c<sup>+</sup> cells have the potential to mediate inducible nitric oxide synthase-dependent cell death in mammary carcinomas of HER-2/neu transgenic mice

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The development of autochthonous mammary tumors in *HER-2/neu* transgenic mice is facilitated by immune tolerance to the *neu*-transgene. However, appropriate vaccination strategies can initiate immune system-mediated antitumor response by a process that requires IFN- $\gamma$ . We investigated the role of inducible nitric oxide synthase (iNOS) induction by IFN- $\gamma$  to promote tumor cell apoptosis. Tumors from FVBN202 mice expressing the normal *neu* gene under the control of the MMTV-LTR were treated in slice cultures with IFN- $\gamma$  for up to 24 hr. Apoptosis was induced, which depended on iNOS enzymatic activity. iNOS expression was predominantly found in infiltrating CD11b<sup>+</sup>CD11c<sup>+</sup> myeloid cells and at much lower levels in the tumor epithelium. By contrast, IFN- $\gamma$  treatment of explant cultures of tumor epithelial cells was not sufficient to efficiently induce iNOS, emphasizing an important role of the integrity of tumor tissue architecture, which was preserved in the slice cultures. This notion was further supported by the upregulation of iNOS costimulatory cytokines TNF- $\alpha$  and IL-1 $\beta$  in slice cultures but not in explants and the capability of purified CD11b<sup>+</sup>CD11c<sup>+</sup> cells to enhance iNOS expression of tumor cells in cocultures. The findings suggest that tumor-infiltrating myeloid cells in immuno-tolerant *HER-2/neu* transgenic mice possess tumor killing ability *via* induction of iNOS and underline the capacity of antitumor strategies designed to stimulate infiltrating myeloid cells.

Interferons play a major role in the dynamic relationship between development of cancer and response of the host immune system. In particular, IFN- $\gamma$  has been shown to be instrumental in cancer immunosurveillance,<sup>1</sup> and vaccination-induced cancer immunoprevention,<sup>2</sup> or immunotherapy.<sup>3</sup>

A key event triggered by IFN- $\gamma$  with potent effects on tumor biology is the production of nitric oxide (NO) *via* induction of the inducible NO synthetase (iNOS).<sup>4,5</sup> iNOS can exert tumor-promoting and tumor inhibitor functions, depending on the stage of tumor development and the amount of NO produced. At low levels, NO acts as an intracellular second messenger and can promote tumor vascularization and serve as tumor promoter, whereas high levels of NO promote nitrosylation of proteins and DNA and can induce cell-cycle arrest and apoptosis.<sup>6-9</sup> Several reports indicate that at least in certain tumor subtypes, iNOS is expressed by the tumor epithelium and expression levels can be linked to either good<sup>8</sup> or bad tumor patient prognosis.<sup>10-12</sup> Furthermore, tumor-infiltrating myeloid cells were identified as a major source for iNOS expression and shown to promote iNOS-dependent tumor-regression.<sup>5</sup> Infiltrating myeloid cells are also considered to be essential in immuno-rejection of the tumor after vaccination,<sup>3</sup> and this effect might be as well dependent on iNOS expression. However, in established tumors, iNOS expression or enzymatic activity is frequently suppressed by a variety of mechanisms.<sup>13,14</sup> During tumor progression, infiltrating myeloid cells can change their phenotype and eventually promote tumor growth and immuno-escape.<sup>14-16</sup> One mechanism for this phenotypic switch appears to be downregulation of iNOS expression. Although the levels of iNOS expressed in these cells are low, they were

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**Abbreviations:** EGFR: epidermal growth factor receptor; iNOS: inducible nitric oxide synthase; IRF-1: interferon regulatory factor 1; MACS: magnetic associated cell sorting; MMTV: mouse mammary tumor virus; NO: nitric oxide; STAT: signal transducer and activator of transcription; TECs: tumor epithelial cells

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