Presence and possible significance of immunocytochemically demonstrable metallothionein over-expression in primary invasive ductal carcinoma of the breast


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Abstract. Metallothioneins (MTs) are ubiquitous low-molecular-weight proteins with a high affinity for heavy metal ions such as zinc, copper and cadmium. MT over-expression has been associated with resistance against anticancer drugs. In the present study we investigated 86 cases (45 cases of tumour category pT1 and 41 of category pT2) of routinely fixed and paraffin-embedded primary breast carcinomas immunohistochemically with a monoclonal antibody to an epitope of MT shared by its I and II isoforms. Immunohistochemically demonstrated MT over-expression was found in the invasive components of 7 of 32 pT1 and 17 of 28 pT2 invasive ductal carcinomas, whereas all 26 invasive lobular carcinomas gave weak or negative results. Fourteen of 17 pT2 and 2 of 7 pT1 invasive ductal carcinomas with MT over-expression developed metastases during follow-up with poor prognostic outcome. In contrast only 3 of 11 pT2 and none of the 25 pT1 cases without MT over-expression had a poor clinical course (P < 0.001). It is concluded that MT over-expression is associated with significantly poor prognosis particularly in pT2 invasive ductal breast carcinomas.

Key words: Metallothionein – Breast cancer – Oestrogen receptors – Progesterone receptors – Immunohistochemistry

Introduction

Metallothioneins (MTs) are ubiquitous low-molecular-weight proteins with a high content (approximately 30%) of cysteine, exhibiting a selective binding affinity for zinc, copper and other group II heavy metal ions. Synthesis of MTs is induced in a variety of tissues by these metal ions as well as by endogenous factors such as glucocorticoids, interferon, interleukin-1 and vitamin D3.

MTs have the ability to bind to large quantities of metal ions, which implies an intracellular reservoir or sequestration function for essential or potentially toxic ions such as zinc and copper, respectively. Furthermore MTs play an important role in the detoxification of toxic metals such as cadmium (Friberg et al. 1974; Leber and Miya 1976; Nomiyama et al. 1982; Goering and Klaassen 1984) and possibly in the cellular protection against ionizing radiation and alkylating agent cytotoxicity (for a review, see Nath et al. 1988).

Increased expression of MTs has been implicated in drug resistance expressed by cell lines derived from a variety of cancers (Kelley et al. 1988). Decrease in the cytotoxic activity of certain anticancer drugs has been reported as well as an increased resistance in MT-rich cells during exposure to ionizing radiation. However, the mechanism by which MTs contribute to this protection is still unclear. It has been suggested that sequestration of drugs or their metabolites may prevent the reaction of these compounds with the respective intracellular targets. Another capacity of MTs is to scavenge free radicals which may contribute to their radio-protective effects (Thornalley and Vasak 1985).
In the present study we investigated 86 cases of primary breast cancer immunohistochemically by means of a monoclonal anti-MT (I and II isoforms) antibody and correlated the immunohistochemical findings with the pTNM classification, the clinical outcome, the oestrogen (ER) and progesterone receptor (PR) status, and the histological tumour type of the respective patients.

**Materials and methods**

Randomly selected and routinely formalin-fixed and paraffin-embedded tissues from 100 primary adenocarcinomas of the breast were reclassified independently by three of the authors (K.W.S., A.H., J.M.W.G.). The staging of the tumours was performed according to the UICC recommendations (TNM-classification; Spiessl et al. 1989) for breast cancer. After reclassification the present series comprised 86 cases including 32 invasive ductal and 13 invasive lobular carcinomas of tumour category pT1 and 28 invasive ductal and 13 invasive lobular carcinomas of tumour category pT2.

Balb/c mice (aged 6–8 weeks) were immunized with 100 μg horse metallothionein (mixture of isoforms obtained from Sigma, product no. 4766) in complete Freund's adjuvant. A second booster injection (100 μg metallothionein/mouse) was given in incomplete Freund's adjuvant. Three days prior to spleen removal, a pre-fusion boost was administered by intravenous injection of metallothionein (100 μg without adjuvant). Fusion of spleenocytes with P2-X63Ag8.653 mouse myeloma cells was carried out using 40% polyethylene glycol 1500. Cells secreting antibodies to metallothionein were selected by ELISA using peroxidase-conjugated anti-mouse IgG. Positive cells were cloned by limiting dilution and monoclonal lines were propagated in pristane-primed Balb-c mice. The ascites fluid obtained of these mice was pooled and used without further purification.

The monoclonal anti-MT antibody E9 has been found (Jasani and Elmes 1991) to be immunocytochemically reactive against a conserved epitope shared by I and II isoforms of human, rat and horse MT. The antibody has been used successfully to detect immunoreactive MT in formalin-fixed, paraffin-embedded tissues of rat and human origin (Elmes et al. 1989; Evering et al. 1990; Fuller et al. 1990).

Tissue blocks were cut at a thickness of 4 μm and mounted on chrome-gel-coated glass slides. After dewaxing and rehydrating in a series of alcohols, endogenous peroxidase was blocked with sodium azide, glucose and glucose-oxidase (all obtained from Sigma, Munich, Germany) according to a modified regimen (Hittmair...