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Original contribution

Progression in melanoma is associated with decreased expression of death receptors for tumor necrosis factor–related apoptosis-inducing ligand

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Summary Tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) induces apoptosis in melanoma by interaction with death receptors TRAIL-R1 (DR4) or TRAIL-R2 (DR5) on melanoma cells or resists apoptosis by interaction with decoy receptors TRAIL-R3 (DcR1) or TRAIL-R4 (DcR2). Studies on cell lines suggest that there is a wide variation in TRAIL death receptor expression; however, their expression on excised human melanoma is not well documented. In view of this, we studied death receptor expression on melanomas using monoclonal antibodies specific for these receptors. Immunohistochemical staining for DR4, DR5, and DcR1/DcR2 was performed on formalin-fixed paraffin-embedded sections of 100 cases of primary melanoma, metastatic melanoma, and benign nevi. Percentage expressions of DR4 versus DR5 in benign nevi, primary melanoma, and melanoma metastases were 40% versus 90%, 69% versus 98%, and 55% versus 66%, respectively. There were significant differences in the mean percentage of DR5-positive cells between different groups of melanocytic lesions. Percent expression was higher in thin (≤1.0 mm) compared with thick primary melanoma (88.9% versus 66.9%), and expression was less in subcutaneous metastases (49%) and lymph node metastases (30.6%) ($P < .005$). Expression was also higher in compound nevi (57%) than dysplastic nevi (49%). DcR1/DcR2 was found in 75% of benign nevi, 62% of primary melanomas, and 74% melanoma metastases. The results showed a wide variation in the expression of death receptors for TRAIL between and within primary and metastatic melanoma and a decreased expression on the thick primary melanoma and metastatic melanoma. This suggests that melanoma

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may not respond to treatment with TRAIL unless given with agents that increase the expression of TRAIL death receptors.

1. Introduction

Tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) is a member of the tumor necrosis factor family, such as the tumor necrosis factor α and Fas ligand, which is a type 2 membrane protein that can induce apoptotic cell death in a variety of cell types [1-3]. Tumor necrosis factor–related apoptosis-inducing ligand appears to be particularly important because it can induce apoptosis in a wide range of cultured malignant cells, but not normal tissues [4-8]. The potential importance of TRAIL as an anticancer agent has been supported by studies in animal models showing selective toxicity in transplanted human tumors but not in normal tissues [9,10]. Induction of apoptosis by TRAIL is believed to be mediated by its interaction with 2 death receptors on cells referred to as TRAIL-R1 (DR4) and TRAIL-R2 (DR5) [6]. It is postulated that normal cells are protected from TRAIL-induced apoptosis by their expression of TRAIL-R3 (DcR1) and TRAIL-R4 (DcR2), which lack cytoplasmic death domains and act to sequester TRAIL (decoy receptors, DcRs) or to mediate antiapoptotic signals [3,11].

We have shown previously that 66% of human melanoma cell lines show partial or complete sensitivity to TRAIL-induced apoptosis [4,5], and that apoptosis appears to be mediated by the mitochondrial pathway [12]. The level of apoptosis induced by TRAIL appears to be determined largely by the level of expression of the death receptor DR5 [5]. In subsequent studies, we found that fresh isolates of melanoma cells from surgical biopsies and short-term cultures of melanoma cells were relatively resistant to TRAIL, and that this appeared to be associated with low TRAIL death receptor expression on the cells [13]. These findings raised questions as to whether administration of TRAIL or use of agonistic antibodies [14] against the death receptors would be effective in treatment if death receptor expression was too low to trigger apoptosis.

To obtain further information about death receptor and decoy receptor expression on melanoma cells in vivo, we have carried out studies on formalin-fixed paraffin-embedded sections of melanomas with antibodies known to be reactive with the receptors in formalin-fixed tissue [15]. The results indicate relatively low expression of DR4, decreasing expression of DR5 and DR4 with progression of disease. DcR1/DcR2 expression was not related to the progression of the disease.

2. Materials and methods

2.1. Patients

The archival paraffin tissue blocks of 100 melanocytic tumors excised and accessioned during the period from 2000 to 2003 were retrieved from the Department of Anatomical Pathology at the Royal Prince Alfred Hospital, Sydney, New South Wales, Australia. There were (1) 10 compound nevi, (2) 10 dysplastic nevi, (3) 42 primary cutaneous melanomas including 18 thin primary cutaneous melanomas (≤1.0 mm in thickness) and 24 thick primary cutaneous melanomas (>1.0 mm in thickness), (4) 21 subcutaneous (SC) melanoma metastases including 2 cases in fibroadipose tissue and 1 case in skeletal muscle, and (5) 17 lymph node (LN) metastases. The 42 patients with primary cutaneous melanomas were treated at the Sydney Melanoma Unit, Sydney, New South Wales, Australia, from December 2000 to March 2002. Hematoxylin and eosin–stained sections of all 100 cases were reviewed. Primary melanomas were classified into thin melanoma (≤1.0 mm) and thick melanoma (>1.0 mm) according to their Breslow thickness. The 42 primary melanomas consisted of 18 nodular melanomas (NMs), 14 superficial spreading melanomas (SSMs), 4 desmoplastic melanomas, 3 in situ melanomas, 2 acral lentiginous melanomas, and 1 lentigo maligna melanoma. All patients with primary melanoma had regular follow-up at the Sydney Melanoma Unit (mean duration of follow-up, 43.5 months; range, 21-50 months).

2.2. Immunohistochemistry

Five-micrometer-thick sections were cut from the formalin-fixed paraffin-embedded tissue block of each case. Sections were deparaffinized in xylene and rehydrated through graded decreasing concentrations of alcohol. Antigen retrieval was performed by adding EDTA, pH 8.6, buffer and by heating in a microwave 3 times for 5 minutes each time [16,17]. Mouse antihuman monoclonal antibodies against DR4, DR5, and DcR1/DcR2 were kindly supplied by Dr Gavin Screaton (Imperial College, Du Cane Road, London) and are described elsewhere [15]. The antibody against the decoy receptors recognized a determinant common to both DcR1 and DcR2. Monoclonal antibodies were incubated on the sections at room temperature for 1 hour at 1:60 (DR4), 1:200 (DR5), and 1:50 (DcR1/DcR2) dilutions in Tris buffer. The Vectastain ABC kit (Vector Laboratories, Burlingame, Calif) was used to detect the monoclonal antibodies according to the manufacturer's instructions, and the binding sites were visualized using the DAB kit (DAKO, Carpinteria, Calif). The sections were counterstained with Harris hematoxylin. Normal mouse IgG was used as a negative control. Sections of colonic adenocarcinoma [18], normal thyroid [15], and positively staining melanomas were used as positive controls.

The slides were examined by 2 pathologists (L. Zhuang and R. A. Scolyer). The percentage of positive cells was estimated from 0% to 100%. Samples showing 10% or less