Letter to the Editor

WNT pathway in laryngeal squamous cell carcinoma and nasopharyngeal carcinoma

Proteine WNT nel carcinoma squamocellulare della laringe e nel carcinoma del rinofaringe

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WNT proteins are a large family of secreted glycoproteins activating the WNT-pathway. WNT binding to Frizzled (Fz) results in the activation of Dishevelled (Dsh), which inhibits the activity of GSK3-β, resulting in dephosphorylation and stabilization of β-catenin, enabling it to accumulate within the nucleus, where it interacts with members of the T-cell factor/lymphocyte enhancer factor (TCF/LEF) family of trans-cription factors to stimulate the expression of target genes. In summary, the canonical pathway translates a WNT signal into the transient transcription of a TCF/LEF target gene programme. Nuclear β-catenin then interacts with various transcription factors to cause cellular proliferation and differentiation. There are several WNT-antagonists that may be classified in two types: a) those that interfere with WNT activity by binding to low-density lipoprotein receptor-related proteins (LRP-5 or LRP-6), including Sclerostin and Dickkopf (DKK) proteins, and b) those that interact directly with WNT proteins, including WIF-1. Although the role of the WNT pathway in nasopharyngeal carcinoma (NPC) has not been fully explored, there is abundant evidence that aberrant WNT signalling is involved in its development [1 2]. Very little data is available on this pathway in laryngeal squamous cell carcinoma (LSCC) [3-5]. Cytoplasmic β-catenin plays a major role in the normal cell by binding to the intracellular domain of E-cadherin to maintain cell-cell adhesion. The expression of E-cadherin has been found to be down-regulated in many cancers including nasopharyngeal carcinoma [2 3]. It has been suggested that E-cadherin down-regulation may play a role in tumour progression and metastasis. Strong β-catenin expression is significantly associated with invasion and metastasis of carcinomas of the head and neck, oesophagus, stomach, colon, liver, lung, breast, female genitalia, prostate, bladder and pancreas, as well as melanoma. Recently, several studies have pointed to the considerable involvement of β-catenin, not only in malignant transformation, but also in the regulation of physiological functions, and expression of this adhesion molecule in human nasopharyngeal carcinoma has been investigated [6 7]; however, it has not yet been thoroughly explored in LSCC.

We sought to evaluate the expression of WNT pathway activators (Wnt-1, Wnt-5a) and inhibitors (WIF-1 and Dkk-1) in tissues from patients with LSCC and NPC and, for purposes of comparison, in patients with non-tumour pathologies. Expression was determined by immunohistochemical analysis using paraffin-embedded specimens from 16 LSCC patients (12 men, 4 women; age 46-72), 18 NPC patients (11 men, 7 women; age 44-78); 11 non-neoplastic nodule specimens (6 men, 5 women; age 19-97) were assayed for control purposes. Immunohistochemistry (IHC) was performed using the peroxidase-antiperoxidase technique. Staining for Wnt-1 (1:100), Wnt-5a (1:200), WIF-1 (1:200) and Dkk-1 (1:250) from Abcam (Abcam, Cambridge, UK) was studied on NPC and LSCC tissues. Histological evaluation was performed by two pathologists, who independently scored the results of immunohistochemical staining; any discrepant scores were re-examined to arrive at a consensus score. Human breast tumour was used as positive control, and negative controls were obtained by replacing the primary antibodies with PBS.

Surprisingly, none of the tissues tested (tumour tissues regardless of location, and non-tumour tissues) exhibited immunoexpression of the WNT pathway activators Wnt-1 and Wnt-5a, whereas all tissues stained positive for the pathway inhibitors, WIF-1 and Dkk-1, displaying similar levels of expression. These findings would suggest that the WNT pathway is inactive in these types of tumours. Earlier research failed to detect nuclear β-catenin, suggesting that the canonical WNT pathway may be inactivatedin both NPC [8] and LSCC (data not shown). However, this cannot be categorically confirmed, since stabilized β-catenin was detected in the cytoplasm. Goiliomus et al., in a study of 97 LSCCs, detected nuclear β-

catenin in some samples [9], perhaps due to differences in tissue processing or to the immunohistochemical staining method used.

Although this hypothesis appears to be confirmed by the present findings, further research is required to determine whether the WNT pathway is activated by overexpression of its receptors or by the silencing of its suppressors. A Western blot could be used for this purpose, with a view to measuring possible alterations in protein levels in fresh tissue, and to investigate the possible activation of the non-canonical WNT pathway that includes signalling through calcium flux, JNK and heterotrimeric G proteins.

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