Pergamon

European Journal of Cancer Vol. 33, No. 4, pp. 635–637, 1997

Printed in Great Britain
0959-8049/97 $17.00 + 0.00

PII: S0959-8049(97)00010-5

Point of View

Homeobox Genes: Molecular Link Between Congenital Anomalies and Cancer

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Homeobox-containing genes play a major role in the control of segmental identity during embryonic development in *Drosophila*. Abnormalities of these genes have been shown to produce a wide variety of congenital anomalies in invertebrates and in vertebrates. Many transgenic mice, which are mutant for homeobox genes, show a specific skeletal abnormality, similar to the human cervical rib. In humans, a relationship exists between malformations and tumours. Human cervical rib has been shown to be associated with an increased incidence of malignancy. Recent evidence indicates that homeobox genes might also play a role in carcinogenesis. In this article, we explore the possibility that alterations of homeobox genes might be the basic underlying aetiology for the association between congenital malformations and tumours, at least in a proportion of cases. We provide evidence in support of this argument and suggest areas of further research which would confirm this concept. © **1997 Elsevier Science Ltd. All rights reserved.**

Key words: homeobox genes, congenital defects, cervical rib, cancer

Eur J Cancer, Vol. 33, No. 4, pp. 635–637, 1997

INTRODUCTION

A RELATIONSHIP exists between malformations and tumours in general as well as in particular combinations. A study of U.S. death certificates revealed an association between congenital malformations and childhood cancers [1]. Examination of a large group of children with malignancy showed a significantly higher incidence of minor anomalies in children suffering from leukaemia and lymphoma [2]. The increased prevalence of minor anomalies in childhood malignancy has also been confirmed by a detailed matched-pair control study [3]. A significantly higher prevalence of supernumerary nipples was seen with renal neoplasms [4]. Subsequently, this association was found in all types of genito-urinary (kidney, prostrate, urinary bladder and testis) malignancies [5]. Congenital malformations of the genito-urinary organs are often associated with Wilms' tumour, giving rise to syndromes such as WAGR syndrome, Denys–Drash syndrome and Beckwith–Wiedemann syndrome. Several authors have reported an association between congenital neuroblastoma and congenital heart disease [6, 7]. Children with Down's syndrome have a higher incidence of acute leukaemia and those with congenital pyloric stenosis show an increased incidence of malignancy [8].

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Received 29 Mar. 1996; revised 2 Oct. 1996; accepted 23 Oct. 1996.

AETIOLOGY OF THE ASSOCIATION BETWEEN MALFORMATIONS AND MALIGNANCY

The molecular lesions responsible for the association of congenital anomalies and tumours need to be explored in detail. Although a consistent chromosomal abnormality (trisomy 21) is reported in Down's syndrome, the molecular lesion underlying the malformation or the associated acute leukaemia is not known. It has been clearly documented that heterozygous deletions/null mutations of the *WT1* gene can lead to both Wilms' tumour and developmental defects of the gonads and kidneys [9, 10]. It is interesting to note that *WT1* expression is observed in kidney as well as in different organs of the genito-urinary system during development [11]. Thus, it is conceivable that genes involved in development could contribute to both tumours and associated malformations. Here we explore the possibility that Class 1 homeobox-containing genes (*Hox* = mice; *HOX* = human) might be yet another family of genes responsible for the association of congenital anomalies and tumours.

HOX GENES AND DEVELOPMENTAL ANOMALIES

Homeobox-containing genes play a major role in the control of segmental identity during embryonic development in *Drosphila* and regulate the expression of other genes during early development [12, 13]. They are highly conserved during evolution and are found also in mouse and man [14, 15]. Abnormalities of these genes have been shown to produce a wide variety of congenital anomalies in invertebrates as well as in vertebrates. These genes show temporal and spatial restriction of expression during embryonic development of mammals as they do in *Drosophila*, suggesting that *Hox* genes may play an analogous morphogenetic role in the development of mammals [16, 17]. Of particular interest is that a number of transgenic mice, which are mutant for *Hox* genes, show a specific skeletal abnormality known as posterior transformation of the seventh cervical vertebra. In these cases, the seventh cervical vertebra develops a pair of ribs and displays a structural resemblance to the first thoracic vertebra. This bone malformation has been observed in transgenic mice with mutation in *Hox*a-4 [18], *Hox*a-5 [19] and *Hox*a-6 [20] genes. We feel that this might represent an analogous bone malformation seen in humans, i.e. the cervical rib, which is seen in 2–6% of individuals [21].

These transgenic mice could serve as an useful animal model to explore the pathogenesis underlying the human cervical rib.

CERVICAL RIB AND MALIGNANCY

Until recently, cervical rib was considered as an incidental or casual finding without much significance. However, a review of chest roentgenographs of 1000 children with malignancies and 200 patients with mainly infectious disease showed a higher incidence of rib anomalies in the former [21]. Rib anomalies were found in 21.8% of the children with tumours and in 5.5% of the control group, which was statistically highly significant ($P < 0.001$). Tumours that are associated with cervical rib in children include neuroblastomas, brain tumours, soft tissue sarcomas, leukaemia and Wilms' tumour. It is interesting to note that the organ/tissue of origin of most of these tumours (neural tissue, kidney* and haematopoietic cells), are also known to express a number of *HOX* family of genes including *HOX*a-4, a-5 and a-6 or their corresponding counterparts during development and differentiation [22-24], and mutations in these genes produce cervical rib-like conditions in transgenic mice.

HOX GENES AND MALIGNANCY

Recent evidence indicates that *HOX* genes might also play a role in carcinogenesis. Within the mouse mammary epithelium, *Hox*c-6 is expressed at low levels in the precancerous tissue, but is not expressed in cancers. In contrast, *Hox*a-1 is expressed only in cancers, not in normal gland or in precancerous mammary tissues, suggesting that *Hox* genes may play a role in a late stage during the development of mammary malignancies [25]. Mouse myeloid leukaemias, which are characterised by frequent deletion in chromosome 2, show a deletion of one of the *Hox*4.1 (*Hox*d.3) genes. It is suggested that deletion of this *Hox* gene plays a role in determining the abnormal developmental programme in mouse myeloid leukaemia [26]. *Hox* genes are also incriminated in a number of human malignancies. Altered expression of *HOX* proteins is seen in tumours of the stomach, colon, breast and testis [27]. In renal carcinomas, genes of group 10 display a marked difference in their transcript when compared to those of normal kidney. *HOX*-2A and *HOX*-2E, which are actively expressed in normal kidney, are absent in cancer biopsies. The *HOX*-3H gene is not expressed in normal kidney, whereas the *HOX*-3H transcripts are present in renal carcinomas [28]. These findings suggest an association between altered *HOX* gene expression and kidney cancer. Alterations of *HOX* gene expression have also been shown in primary colon cancers and their hepatic metastasis, suggesting an association with colon cancer progression [29]. Small cell lung cancer (SCLC) of different histology and grade display differential patterns of *HOX* gene expression. Furthermore, in SCLC, the number of actively expressed *HOX* genes might be substantially lower in metastatic cancers than in primary tumours. Thus, downregulation of *HOX* genes may play a role in SCLC progression, possibly through their implication in tumour suppression [30]. *HOX* genes are known to be involved in translocations in acute leukaemias in human. The *HOX-11* (*TCL-3*) gene has been cloned through the studies of the t(10;14) chromosomal translocation found in the T-cell acute lymphoblastic leukaemia [31–34]. Molecular analysis revealed that the majority of the t(10;14) chromosomal translocation break points occurred in the DNA region upstream from the 5′ end of the *HOX-11* gene, indicating that the chromosomal translocation is responsible for deregulation of the *HOX-11* gene observed in leukaemic T-cells [35–39].

MOLECULAR LINK BETWEEN CONGENITAL ANOMALIES AND CANCER

It is possible that abnormalities of homeobox genes might be the underlying molecular lesion, at least in a proportion of cases, in which congenital anomalies are associated with cancer. This concept is based on the following evidence: (1) *Hox* genes are shown to be responsible for many congenital anomalies in animals; (2) *HOX* genes are implicated in many animal and human tumours; (3) some *Hox* gene alterations in mice produce transformation of seventh cervical vertebra, which is analogous to the human cervical rib; (4) cervical rib in man is associated with a number of malignancies. The organ/tissue of origin of these tumours also expresses the homologous or paralogous genes, abnormalities of which produce cervical rib-like anomalies in mice; (5) it is possible that abnormalities of *HOX* genes might play a causative role in both the rib anomaly and cancer found in these cases, and this may also be true for at least a proportion of other congenital anomalies associated with cancer.

CONCLUSION

Further work in two areas would strengthen the above concept. It is essential to identify *HOX* gene abnormalities in cervical rib patients. Given the complexity of paralogous *HOX* genes and the fact that multiple *HOX* gene abnormalities could give rise to similar rib anomalies, no doubt it would be tedious. Furthermore, it is possible that abnormalities in different *HOX* genes would produce the same cervical rib anomaly, but the associated malignancy could differ

*Data based on *Hox* gene expression in mouse kidney.

depending on the individual *HOX* gene(s) involved. It is also important to study the incidence of spontaneous or induced cancers in transgenic mice with mutations in *HOX* genes. To our knowledge, no studies have been published which address these issues, and we believe that future research in these directions would yield valuable information.

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