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Neuroblastoma: A Multiple Biological Disease

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Neuroblastoma (NB) is a paediatric tumour showing an appreciable variability in clinical evolution. Localised tumours (especially stage 1) can be mildly treated with good success while metastatic tumours (stage 4) are highly aggressive. This suggests a great biological diversity. In fact, molecular and genetic studies have revealed distinct abnormalities in localised and non-localised tumours. Loss of heterozigosity for the short arm of chromosome 1, 1p deletion, and MYCN amplification are present in stages 3 and 4 but rarely in stages 1 and 2. Metastatic stage 4S in infants is peculiar and does not show the same genetic and molecular abnormalities found in advanced metastatic tumours. Considering the biological alterations associated with NB, it would appear that advanced stage NB conforms to the multistep model of tumour development while stage 4S can be divided into two groups: one arising from a lack of cellular differentiation and the other as a consequence of an additional 'one hit' mutation.
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HUMAN NEUROBLASTOMA is clinically characterised by a complex evolution showing different degrees of aggressiveness. Six clinical stages (including 1, 2A, 2B, 3, 4 and 4S) have been identified [1]. Patients in stages 1 and 4S receive minimal or no therapy while stages 2A-B receive polychemotherapy; moreover, stages 1 and 2A-B may each be managed surgically. The prognosis of stages 1, 2A-B and 4S is good with a long survival. On the contrary, a poor prognosis identifies patients in whom the tumour infiltrates across the midline of the body to affect several lymph nodes (stage 3), with potential dissemination of neuroblasts to distant organs (stage 4). In addition, patients in stages 3 and 4 who receive several cycles of chemotherapy show a minimal survival at 2 years from diagnosis. This dramatic difference between the two sets of patients (stages 1, 2A-B, 4S and stages 3, 4) may be underlined by intrinsic biological differences. Indeed, several studies have shown differences in chromosome content, gene expression and other biological and molecular aspects. Chromosome 1p deletion found in neuroblastoma is restricted to cells of stages 3 and 4, while only rarely has this been observed in other stages [2]. However, chromosomal microdeletions and loss of important 1p gene(s) in stage 1, 2 and 4S cells cannot be excluded. Fong *et al.* [3] and Weith *et al.* [4] have identified the locus for a putative neuroblastoma suppressor gene. Unlike Weith, Fong and co-workers demonstrated a significant association between loss of 1p genes and MYCN amplification. MYCN oncogene has been shown to be amplified in stages 3 and 4 neuroblastoma and MYCN amplification correlates with tumour progression. Occasional cases of stages 1, 2 and 4S with gene extra copies of MYCN have also been noted, however [5–9]. Other reports have described MYCN gene expression variously distributed throughout different stages, without a clear correlation with prognosis [10].

Most stage 4 tumour cells are usually 'near-diploid' [11, 12]. Patients in stage 4 show a poor prognosis in relation to patients bearing near-triploid or hypotetraploid tumours [13–15]. Neuroblastoma cells may also contain double minute chromosomes (DM) or chromosome regions which stain homogenously (HSR). Other relevant observations include frequent loss of heterozigosity of chromosome 14q and chromosome 11 [16,17]. The major abnormalities found in neuroblastoma cells are listed in Table 1.

As mentioned above stage 1 and 2 are characterised by non-metastatic neuroblastic elements, while in stage 4S which appears in infants less than 6 months of age, they grow as a localised mass and metastasise the body. Apart from rare exceptions (10–21%), stage 4S patients have a good prognosis and, within a few months of diagnosis metastases disappear [18–20]. There is evidence that regression is the result of spontaneous cell maturation [20]. Thus, the capacity to metastasise and to mature distinguish 4S cells as a diverse biological entity. Cell culture and cell transplantation in nude mice cannot be achieved using 4S-stage cells, though they are successful with 4-stage cells. Furthermore, the dosage of neurone-specific enolase, a marker of disease progression and metastatic process, was found to be low in stage 4S (as well as in localised neuroblastoma) but high in stage 4 [21].

The neuroblastoma cell appears to be the result of a multistep evolution where additional mutational events are accumulated. Like the "long route" leading to the colorectal carcinoma cell transformation [22], the neuroectodermal cell seems to accumulate successive critical changes. Possibly the first is the loss of 1p material followed by DM formation and HSR, followed by gene amplification or vice versa. MYCN amplification could

Table 1. Major genetic abnormalities found in neuroblastoma

Stage	1p*	11q†	14q†	MYCN‡ gene	Near diploid	Aneploidy	DM/HSR
4S	−	?	−	−	+	−	
1	−	−	−	−	+	−	
2	−	−	−	−	+	−	
3	+	+	+	+	−	+	
4	+	+	+	+	−	+	

* Loss of genes localised in the region 1p-->ter.
† Loss of heterozigosity of chromosome 11q or 14q.
‡ Amplification of MYCN gene. MYCN mRNA expression can be present or not associated to MYCN gene amplification; there is no clear correlation with the stage.

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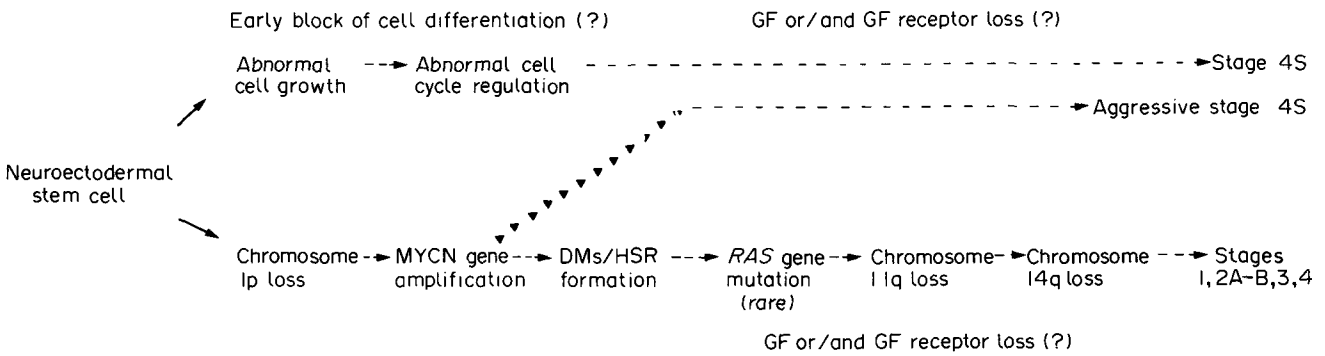

Fig. 1. Possible route of neuroblastoma development.

occur as a later event. However, at present the order of the mutational events cannot be determined. Figure 1 shows a diagram of possible events participating in the transformation of the neuroectodermal stem cell. Additional events could be *ras* point mutation, which has been reported but is very rare [23], and also 14q and 11q allelic loss.

Conversely to stages 1 to 4, 4S cells could be the product of abnormal differentiation pathway and this may be indirectly supported by the absence of the classical genetic alterations of neuroblastoma in 4S cells. If this were the case, stage 4S could be characterised by an intrinsic defect of differentiation such as a loss or abnormal function of growth factor(s) and/or growth factor receptor(s). Knudson and Meadows have hypothesised that 4S is not a malignant tumour, but rather a hyperplastic nodule of mutant cells lacking a second event present in typical neuroblastoma cells [24]. However, Frantz [25] suggests a humoral regulation hypothesis in which a humoral factor delays the normal cell differentiation. This could also be supported by the evidence that patients in stage 4S can show complete remission after mild chemotherapy. In fact, it is known that antineoplastic drugs are able to induce cell differentiation and reversion of malignant phenotype in many *in vitro* cell models [26–29]. Thus, complete remission observed in these patients could be the result of induction of terminal differentiation.

However, it is impossible to exclude completely that 4S cells are the result of a one-hit mutation. This hypothesis could be supported by the fact that at least 18% of stage 4S show MYCN oncogene amplification [7,30,31].

From this we might suggest that stage 4S could be divided into one-hit mutated 4S and undifferentiated 4S. The one-hit mutated 4S is associated with the alternative stages of neuroblastoma while undifferentiated 4S cells remain a distinct entity in which a mutational event has yet to be demonstrated.

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Modalities Available for Screening for Prostate Cancer

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INTRODUCTION

IN THE EUROPEAN COMMUNITY (E.C.) carcinoma of the prostate is the second leading cause of cancer deaths among men after carcinoma of the lung [1]. The death rate was 22.6 per 100 000 men during the years of 1980–1984 (9% of all male deaths of cancer). Within the E.C. 85 000 new cases are diagnosed per year. In the U.S.A. prostate cancer has become the most commonly diagnosed cancer in males. In 1992, 132 000 new cases are expected. Over a 13-year period from 1973 to 1985 the age-adjusted incidence of prostate cancer has increased at a rate of 2.2% per year in the U.S.A. The age-adjusted mortality increased 0.8% annually [2]. In the Germany, France, Belgium and the Netherlands the age-adjusted mortality is similar to that of the U.S.A. In comparison with the U.S.A., the number of deaths due to prostate cancer in the Netherlands has increased by 2% annually over the past 10 years [3]. One explanation for this increase lies in the fact that men now live longer, to an age at which prostate cancer becomes sufficiently advanced to cause death. Moreover, the reporting of the disease may be increased [4]. The increase of prostate cancer deaths is poorly understood. Possibly, after correction for these factors the mortality would not appear to increase at all.

Unfortunately, at the time of initial presentation most patients exhibit evidence of advanced disease. Curative treatment of prostate cancer is only possible if the disease is detected before metastases are present and the cancer is still confined to the prostate gland.

The high mortality on one hand and the availability of apparently effective treatment of locally confined disease by surgery on the other warrants reconsideration of early detection studies. For this reason the availability of accurate screening tests is a prerequisite. Digital rectal examination (DRE), prostate-specific antigen (PSA) and transrectal ultrasonography (TRUS) are available. The question is: are these tests suited for early detection studies and screening programs?

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DEFINITIONS

When discussing screening studies it is important to understand statistical terms often used [5]. The term 'sensitivity' of a test refers to the number of patients who actually have the disease and in whom the test is positive, divided by the total number of patients with the disease. The term 'specificity' refers to the number of men who actually do not have the disease and in whom the test is negative, divided by the total number of men without the disease. The term 'positive predictive value' (PPV) is the probability that the disease is, in fact, present given a positive test result.

The PPV is strongly influenced by the prevalence of the disease. Patients who come for evaluation in a urological practice probably are not representative of a general population. Therefore, a description of the group of males which was subject to screening or early detection is needed for a sensible comparison of different studies.

The detection rate is the proportion of those who are detected as having the disease within the total screened population.

DIGITAL RECTAL EXAMINATION

DRE is the classic technique for the detection of prostate cancer. The prostatic structures such as apex, basis, median and lateral sulci and the seminal vesicles can be readily palpated transrectally. Rough estimates of the size of the prostate and of lesions within the prostate can be made in a bidimensional fashion. Prostate cancer, if it is confined, will appear as a discrete induration or a nodule (T2). More extensive prostate cancer appears diffusely firm and extends beyond the lateral sulci or into the seminal vesicles (T3). Fixation at the pelvic wall will usually occur at the level of the levator ani muscles (T4). Incidental prostatic cancer (T1) is found in 8–12% of cases when tissue removed for the treatment of benign prostatic hyperplasia (BPH) is examined histologically [6].

If a prostate is suspicious for prostate cancer a biopsy must be taken. Prostatic cancer is confirmed in 22–39% of such cases (Table 1). The differential diagnosis includes BPH, prostatitis, granulomatous prostatitis, prostatic calculi and tuberculosis.

In the 1950s DRE was used by Jensen *et al.* [7] in a screening program involving 4367 asymptomatic men. Over a 10-year period, 36 men were found to have carcinoma of the prostate,