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Editorial Comment

MYCN amplification remains prognostically strong 20 years after its "clinical debut"

Susan L. Cohn [a], Deborah A. Tweddle [b,*]

[a] *Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA*
[b] *Northern Institute for Cancer Research, Medical School, University of Newcastle, Framlington Place, Newcastle-upon-Tyne NE2 4HH, UK*

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More than 20 years ago, Schwab and colleagues [1] identified multiple copies of the *MYC*-related oncogene, *MYCN*, in a panel of neuroblastoma cell lines and a neuroblastoma tumour. Subsequently, *MYCN* amplification was shown to occur in approximately 22% of primary neuroblastomas in untreated patients and to have a significant association with advanced-stage disease, rapid tumour progression and poor prognosis [2,3]. However, only one third of stage 4 neuroblastomas over 1 year of age have amplified *MYCN* so there must be other unfavourable molecular abnormalities accounting for the inferior survival, at best 30–40% at 5 years, in the remainder of this group. *MYCN* is normally located on the distal short arm of chromosome 2 (2p), but extrachromosomal double-minutes or homogeneously staining regions are the chromosomal sites of amplified *MYCN* [4]. To date, the molecular mechanisms of amplification remain an enigma. The *MYCN* amplicon can range from 100 kb to more than 1 Mb, but a core 100- to 200-kb domain encompassing *MYCN*, without rearrangement, is consistently seen [5]. Although other genes are co-amplified with *MYCN* in some cases, *MYCN* is the only gene that is consistently amplified from this locus [6].

The reason why *MYCN* amplification is associated with a more aggressive phenotype is still uncertain. The *MYCN* gene encodes a 60-63 kDa nuclear phosphoprotein that contains a basic helix–loop–helix/leucine zipper (bHLH-LZ) motif [7]. Like other members of the MYC family, MYCN is a transcriptional regulator that appears to play a critical role in cellular proliferation, differentiation, transformation, and apoptosis [8]. For MYCN to activate transcription, it must first dimerise to MAX, another bHLH-LZ protein [9]. The specificity of MYC transactivation is partly mediated through binding to a core E-box promoter sequence, CAT/CGTG, found in the promoter region of target genes. Several genes including ornithine decarboxylase (*ODC*), *MCM7*, and multidrug-resistance associated protein 1 (*MRP1*) are upregulated by MYC proteins [10–14]. However, the *MYCN* target genes critical for neuroblastoma genesis and tumour progression have not yet been defined.

Tumours with *MYCN* amplification usually express *MYCN* at high levels, and this subset of tumours are clinically aggressive. High levels of *MYCN* expression also clearly enhance malignant neuroblastoma growth in laboratory studies, whereas *MYCN* antisense inhibition is associated with decreased cell proliferation and neuroblastoma tumour growth [15–17]. A role for *MYCN* in neuroblastoma pathogenesis is also supported by studies demonstrating neuroblastoma tumour development in transgenic mice with targeted expression of *MYCN* [18]. However, the clinical significance of increased *MYCN* expression in children with neuroblastoma is still controversial [19–21]. The reason for these discordant results may, in part, be due to disparities in patient populations, as the proportion of infants <1 year of age, patients with advanced-stage disease, and those with *MYCN*-amplified tumours differs in the various series. Recently, high levels of *MYCN* expression were found not to be predictive of a worse outcome in a retrospective analysis of patients with advanced-stage disease and normal *MYCN* copy number [22]. Thus, the precise role, if any, that *MYCN* plays in non-amplified tumours is still unknown.

Worldwide, the current treatment for older children with local-regional neuroblastoma and infants with both

* Corresponding author.
E-mail addresses: scohn@northwestern.edu (S.L. Cohn), d.a.tweddle@newcastle.ac.uk (D.A. Tweddle).

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regional and invasive or disseminated disease is stratified on the basis of *MYCN* amplification status. Patients with non-amplified tumours in these groups receive surgery with or without moderate-dose chemotherapy. Much more intensive multi-modal therapy, including dose-intensive induction chemotherapy, surgery, myeloablative consolidation therapy and haemopoietic stem cell rescue, radiotherapy and differentiation therapy with retinoids, is used to treat patients with amplified tumours in an attempt to improve outcome. Clearly, because the treatment for patients with *MYCN*-amplified versus non-amplified tumours is so different, it is of paramount importance to measure *MYCN* amplification accurately and reliably. In Europe, the quality of *MYCN* amplification measurement is assured because analyses are performed in approved national reference centres under the auspices of the European Neuroblastoma Quality Assurance Group [23]. Similarly, a single Clinical Laboratories Improvement Amendments (CLIA) approved reference laboratory is used in the Children's Oncology Group (COG) to evaluate tumour cell *MYCN* amplification.

Southern blot analyses were initially used to measure *MYCN* copy number, and although this methodology requires relatively large amounts (10 μg) of DNA and a tumour cell content of >60%, the technique still represents the 'gold standard' for quantification of *MYCN* copy number. Over the past 15 years, fluorescent *in situ* hybridisation (FISH) has been developed as an alternative technique. FISH has several advantages over Southern blotting because (i) it can be performed on very small amounts of tumour tissue including imprints, bone marrow smears and paraffin tumours, (ii) focal or heterogeneous amplification can be detected and (iii) DNA ploidy can be determined. More recently, a quantitative polymerase chain reaction (PCR) technique has been developed but, like Southern blotting, it requires a tumour cell content in tested samples of >60%, so FISH is always recommended as a supplementary technique [23]. Neuroblastomas lacking amplification do not necessarily have the normal single copy of *MYCN* per haploid karyotype. Some tumours contain 1–4 extra copies of the *MYCN* gene relative to chromosome 2 copy number. This phenomenon has been defined as '*MYCN* gain'. *MYCN* gain may arise by several mechanisms. Duplication of 2p24 [24] and unbalanced translocation of the distal end of the short arm of chromosome 2 [25] have both been reported, using the FISH technique, in neuroblastoma cell lines (Fig. 1).

In this issue of the *European Journal of Cancer*, Spitz and colleagues [26] report the first systematic survey comparing the clinical impact of neuroblastoma tumours with *MYCN* gain, *MYCN* amplification, or non-amplified *MYCN*. This is a large study in which FISH was utilised to test for *MYCN* amplification in 659 cases. *MYCN* gain was found to be present in 38 (6%) tumours, and was associated with a higher stage of disease, older age, and 11q loss. In contrast to *MYCN* amplification, *MYCN* gain was not associated with increased *MYCN* expression at the RNA level. Inferior event-free outcome was seen in patients with *MYCN* gain, but this genetic abnormality was not predictive of overall survival for children with localised tumours and was not prognostic in metastatic tumours. A previous smaller study of 110 tumours reported *MYCN* gain in 9/110 (8%). In that study, *MYCN* gain was observed with 1p deletion and/or 17q gain in 5/7 cases and increased *MYCN* RNA levels in 3/5 cases. There was no relationship between *MYCN* gain and survival, although the median follow-up time was only 26 months [27].

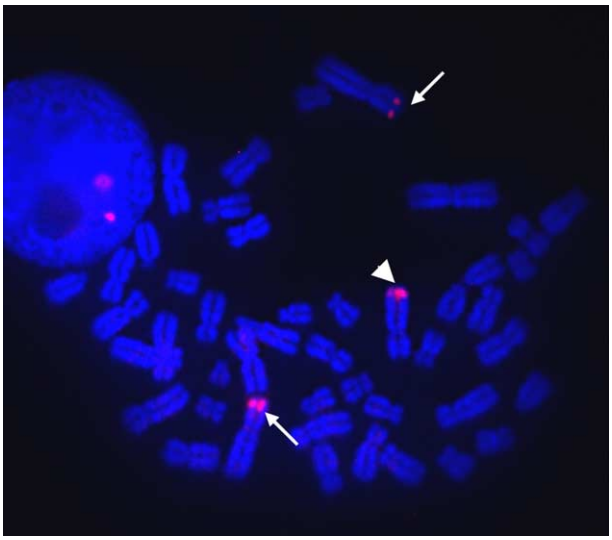

Fig. 1. Metaphase fluorescent *in situ* hybridisation (FISH) showing *MYCN* gain in the NB69 neuroblastoma cell line. One copy of *MYCN* is on each 2p (arrows) and an extra copy of *MYCN* is present on distal der(8)q as an unbalanced translocation (arrowhead).

The other salient features of the paper by Spitz and colleagues relate to confirmation of the prognostic effect of *MYCN* amplification in patients with localised and metastatic neuroblastoma [28,29]. Amongst those with *MYCN*-amplified tumours, infants had a superior overall and event-free survival than older patients, including those in both groups with stage 4 disease. This finding contrasts with previous studies reporting survival of infants with *MYCN*-amplified tumours and stage 4 disease to be similar to that of older patients with stage 4 disease [29]. Spitz and colleagues also observed that patients with *MYCN*-amplified localised tumours had better overall survival than those with *MYCN*-amplified metastatic tumours. Interestingly, *MYCN* amplification was also found to be prognostic in patients with stage 4 disease over 1 year of age. However, it must be remembered that patients in this study were treated on a number of different trials during the time of tumour collection (1989–2003), and treatment is, of course, a powerful prognostic factor.

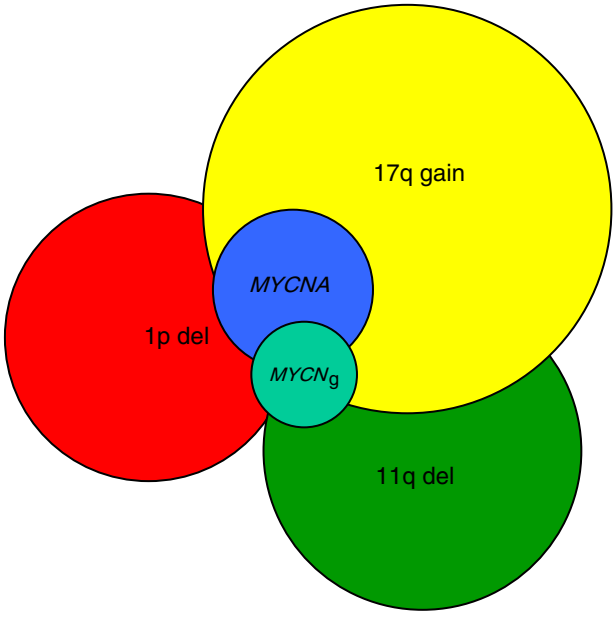

Fig. 2. Inter-relationship of some of the genetic abnormalities in neuroblastoma. Circle size corresponds to approximate frequency of each genetic abnormality. *MYCNA*, *MYCN* amplification; *MYCNg*, *MYCN* gain; del, deletion.

In summary, throughout the last 20 years the presence of *MYCN* amplification has consistently emerged as a marker of adverse prognosis in different subgroups of neuroblastoma. In addition to *MYCN*, a number of other genetic abnormalities, including 1p loss, 11q loss and 17q gain, have been found to have prognostic significance in univariate analyses of neuroblastoma, but, to date, there is no consensus as to which of these is most significant on multivariate analyses. Of special interest is that *MYCN* amplification has never been reported without either 1p loss or 17q gain (Fig. 2). It is therefore apparent that in many cases of neuroblastoma, *MYCN* alone is not sufficient to distinguish those patients who are likely to survive from those that are destined to fail treatment. Further discrimination may be achieved by including 1p loss, 11q loss and 17q gain, but it is likely that more precise risk stratification will also depend on additional information gained from newer molecular techniques, such as gene expression profiling. These techniques are already proving to have significant prognostic potential in a variety of adult cancers and leukaemias [30,31].

Conflict of interest statement

None declared.

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