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N-myc amplification in Neuroblastoma based on fluorescence in situ hybridization (FISH): Correlation with histopathological features

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LIST OF ABBREVIATIONS

BMPs	**Bone Morphogenetic Proteins**
CCD	**Charge-Coupled Device Camera**
CGH	**Comparative Genomic Hybridization**
C-myc	**Cellular Myelomatosis oncogene family**
DAPI	**Diamidino-2-phenylindole**
DM's	**Double Minutes**
DNA	**Deoxyribonucleic acid**
F	**Favorable Histopathology**
FISH	**Fluorescence In situ Hybridization**
FITC	**Fluorescein isothiocyanate**
GD2	**Cell surface ganglioside**
Her-2/neu	**Human epidermal growth factor-2**
HSR's	**Homogenous Staining areas**

HuD	**neuronal-specific RNA-binding protein, is a potential regulator of MYCN expression in human neuroblastoma cells**
HVA	**Homovanillic Acid**
INSS	**International Neuroblastoma Staging System**
LDH	**Lactate Dehydrogenase**
LSI-N-myc2P24-1	**N-myc probe for FISH provided by Vysis company**
Mad	**A heterodimeric partner for Max that antagonizes Myc transcriptional activity**
MASH1	**The proneural gene Mash1 specifies an early population of telencephalic oligodendrocytes**
Max complex	**A helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc**
met`s	**Metastasis**
MKI	**Mitosis–Karryorhexis Index**
MR	**Mitotic Rate**
MRP	**Multidrug Resistance associated Protein**
NB-84	**Anti Neuroblastoma antibody**

NCAM	**Neural Cell Adhesion Molecule (NCAM) as a regulator of cell-cell interactions**
NCI	**National Cancer Institute**
N-myc	**Neuroblastoma derived Myelocytomatosis viral related oncogene**
NSE	**Neuron-specific enolase**
PBS	**Phosphate-Buffered Saline**
PCR	**Polymerase chain reaction**
PFS	**Progression free survival**
Phox2a	**protein plays a central role in development of the autonomic nervous system. It regulates the expression of tyrosine hydroxylase and dopamine beta-hydroxylase essential for the differentiation and maintenance of the noradrenergic neurotransmitter phenotype**
Phox2b	**protein plays a central role in development of the autonomic nervous system. It regulates the expression of tyrosine hydroxylase and dopamine beta-hydroxylase essential for the differentiation and maintenance of the noradrenergic neurotransmitter phenotype**
PI	**Propidium iodide**

PNET	**Primitive neuroectodermal tumors**
Ret	**Rearranged during Transfection Retinoblastoma-like protein 2 RET is mainly expressed in tumors of neural crest origin**
SSC	**Saline Sodium Citrate**
SVV	**Survivin, mapped to 17q25, is significantly associated with poor prognostic factors and promotes cell survival in human neuroblastoma**
TrkA	**Tropomyosin-Related kinase A is expressed preferentially in neuronal tissues**
TrkB	**Tropomyosin-Related kinase b**
TrkC	**Tropomyosin-Related kinase c**
TSG	**Tumor suppressor gene**
TTAGGG	**DNA bases**
UH	**Unfavorable Histopathology**
VMA	**Vanillylmandelic Acid**
LOH	**Loss Of Heterozygosity**
P	**Short arm of chromosome**

Q	**Long arm of chromosome**
Alpha satellite DNA	**Class of repetitive DNAs located at the chromosomal centromere.**
Unique sequence probes	**Probes for specific genes or loci found twice in a diploid cell.**
Whole chromosome probes	**Collection of unique sequence probes covering an entire chromosome**

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INTRODUCTION

Neuroblastic tumors are common pediatric tumors. These tumors are derived from immature sympathetic neuroblasts during embryonic, fetal, or early postnatal development and their morphological features appear to recapitulate developmental stages of sympathetic ganglia. Their primary sites are anatomically related to the embryological distribution of neural crest cells, and include adrenal gland and structures of the sympathetic nervous system **(Shimada and Nakagawa, 2006).**

For many years neuroblastic tumors were characterized as "enigmatic" because of their unexpected clinical behavior, such as involution/spontaneous regression, maturation, or aggressive progression. **(Tang *et al.*, 2006).** Because of advances in clinical and basic research, neuroblastic tumors are considered to be biologically heterogeneous, and their individual molecular properties like account for their unique clinical behavior **(Maris and Matthay, 1999).**

Neuroblastoma is one of the most common cancers affecting children, forming 7%-8% of all pediatric malignant disease. It occurs with slightly more frequency in males than in females. The median age at diagnosis is 2 years **(Berthold and Simon, 2005).** Neuroblastoma typically begins in the abdominal area either in the adrenal gland or around the spinal cord in the neck, chest, or pelvis. Although neuroblastoma often is present at birth, it is generally not detected until the tumor begins to grow and compress the surrounding organs.

Cancer cells can metastasize quickly to other areas of the body, such as lymph nodes, liver, lungs, bones, the central nervous system and bone marrow; close to 70 % of children diagnosed with neuroblastoma will have metastatic disease **(Hsu *et al.*, 2006).**

Neuroblastoma has a wide range of "virulence"; children with localized neuroblastoma can be cured by surgery alone but those with bone metastases usually have a fatal outcome. Neuroblastomas have heterogeneous biologic, genetic, and morphologic features and are characterized by diverse clinical behavior. Although the biological basis for this diversity is poorly understood, many molecular features, such as DNA index, oncogene amplification, and tumor suppressor gene loss, have been identified that correlate with clinically relevant aspects of the disease **(Bown, 2001)**. Because of this strong relationship between biology and clinical phenotype, molecular classification is playing an increasingly important role in stratifying therapy for patients with neuroblastoma **(Simpson and Gaze, 1998).** The identification of tumor-specific molecular alterations and the characterization of critical pathways regulating tumor growth are likely to further refine the ability to diagnose and classify neuroblastoma, and may lead to the identification of therapeutic targets **(Hsu *et al.*, 2006).**

AIM OF THE STUDY

The aim of this study is to evaluate N-myc amplification by FISH method performed on paraffin embedded tissues of neuroblastoma and to correlate the N-myc amplification with the different histopathological features of neuroblastoma tumors and to discuss the importance of N-myc amplification as a prognostic marker in neuroblastoma and its impact on therapy.

I: EPIDEMIOLOGY

In the United States, neuroblastoma accounts for 7.2% of all cancers among children younger than 15 years of age **(SEER, 2003).** It is the most common extra cranial solid tumor of childhood **(Goodman *et al*., 1999).** Based upon 1424 incident cases identified by the Surveillance, Epidemiology, and End Results Program of the USA National Cancer Institute (NCI) for 1975–2000, the total incidence of neuroblastoma was 10.2 per million children under age 15 years **(SEER, 2003)**. The rates were 10.3 per million for males and 10.1 for females. According to Cairo University National Cancer Institute registry neuroblastoma accounted for 2.3 % of pediatric primary malignant tumors **(Mukhtar *et al*., 2007)**.

Based upon international registry data, the incidence of neuroblastoma is highest among Caucasians from North America, Europe, and Australia **(Stiller and Parkin, 1992).** Lower rates were found for registries in southern and eastern Asia, including India and China, and also in Latin America. Overall, the incidence appeared to be higher for regions or ethnic groups with a higher standard of living **(Stiller and Parkin, 1992)**. A previous study by SEER data found no total increase in incidence over time but reported a 3.4% average annual percentage increase for infants diagnosed between 1973 and 1992 **(Gurney *et al*, 1996)**. The average annual increase was twice as high for infant boys as for infant girls. Studies in the United States and elsewhere have noted increases in the incidence of neuroblastoma **(Olshan and Bunin, 2000)**.