The Role of ploidy in neuroblastoma

Michael D. Hogarty, MD
Division of Oncology
Children's Hospital of Philadelphia

Ploidy is a general term referring to the amount of chromosomal material (that houses genetic information in the form of DNA) in cell. Normal human cells all contain the same amount of DNA as two full sets of chromosomes, having received on full set from each parent. These cells are therefore called "diploid" (think 'di' = two sets). Cells duplicate these chromosomes (and other structural materials) each time they divide to provide the cells needed for tissue growth, repair, and maintenance.
Remarkably enough they are able to coordinate their growth so that each daughter cell has an exact copy of the diploid genetic content of the parent cell, and therefore remain diploid with no gain or loss of genetic material. Further, their growth is very tightly controlled so as to limit cell division to times and tissues where extra cells are required. Cells that are damaged or attempt to proliferate autonomously frequently trigger an inherent suicide program leading to their death (1).

Tumor cells are a different story. Though they arise from normal cells they are characterized by many genetic abnormalities (mutations) that lead to rapid growth and loss of normal control. Cancer Cells replicate themselves with wanton disregard for their primary function or the good of the

organism. For example, neuroblasts are unable to function as nerve cells and hepatoblasts do not function as normal liver cells. In many human cancers the amount of DNA in these tumor cells has become abnormal. The cells are no longer diploid but instead have lost or gained DNA during the mutation process. This is generically termed aneuploidy (meaning "without normal ploidy"). Aneuploidy is so pervasive within tumor cells that some have considered it a causal requirement for tumor formation, although most cancer biologists feel aneuploidy is a result rather than a cause of the cancer process. A simple way to think of this is to view cancer cells as being so single-mindedly focused on proliferating that they are reckless in copying their genetic information-they allow many mistakes to be made in their haste. This often leads to grossly abnormal DNA content: broken chromosomes, extra copies of entire chromosomes, loss or gain of parts of chromosomes, etc. Much of this genetic damage continues to benefit the cancer cells by altering more and more genes, many of which function to prevent cancer cell growth when they're intact but can't do so when they're lost of mutated.

Cancer cells that have gained genetic material are called hyperdiploid (meaning "more than diploid"). Examples are triploid and tetraploid cells that have approximately three (tri-) or four (tetra-) sets of chromosomal material, respectively. Conversely, cells that have lost genetic material are referred to as hypodiploid. The actual measurement of ploidy is called the DNA index (DI) or DNA content, with normal diploid cells serving as a reference point. So by definition, a DNA index of 1.0 is 1.0 times the DNA content a normal cells should have, or diploid. Any DNA index over 1.0 is hyperdiploid while those under 1.0 are hypodiploid. Of course, even a cancer cell that is diploid on the basis of its DNA content is not a normal cell with normal DNA. These cells still

have an assortment of genetic mutations that allow them to grow rapidly and escape normal cell control mechanisms, however they are not the kinds of genetic alterations that lead to grossly abnormal DNA content.

None of this would matter to anybody but cancer scientist if the DNA index of a tumor cell didn't have any practical meaning. As it turns out, in many cancers the ploidy of the tumor cells has some predictive value in determining how aggressively the cancer may behave. This is not universally true so it must be investigated in each cancer type. It just so happens that if you examine the ploidy of neuroblastoma cells it helps predict tumor aggressiveness, but this mainly holds true in younger children with widespread disease. Initial reports on the correlation between neuroblast ploidy and outcome reported a significantly better chemotherapy response in infants whose tumors were hyperdiploid rather than diploid or tetraploid (2). Of note, all of these infants had tumors that could not be fully removed surgically. Further studies confirmed that for disseminated neuroblastoma (Stage 4 or 4S) patients whose tumors were hyperdiploid had an improved survival outcome, but this finding was age dependent. Ploidy was highly predictive in children under 1 year of age, whereas between 1 and 2 years of age the importance became less dramatic and for tumors in children over 2 years of age it did not appear to add any additional predictive information (3). So in certain clinical situations dependent on the child's age and tumor stage it can be said that diploid or tetraploid tumors tend to behave badly by being more aggressive and resistant to treatment, whereas tumors in between (called hyperdiploid or near-triploid, but NOT tetraploid) tend to respond well. Thus, ploidy can be considered a prognostic marker for disease behavior.

The question of why tumor cell ploidy gives prognostic information is more difficult. The determination of cell ploidy provides a rather rough measurement of DNA content. Knowing the average amount of DNA per cell tells us nothing about what chromosomal changes may have occurred or what genes may have been activated or inactivated. Instead, these specific chromosome abnormalities can be discovered by detailed analyses of the tumor cells obtained by cytogenetics or a karyotype (4). In these tests the cells are processed in a special way to view the individual chromosomes in their entirety, so even if the overall amount is roughly normal, you can often detect mutations such as broken chromosomes or rearrangements. To complicate things, however, cytogenetic analysis is not easy to do on solid tumors and is not always successful, so it's good to have alternative ways to get the information needed. Likewise, direct molecular tests are required to determine the status of a particular gene (like MYCN copy number) or chromosome region (such as for 1p deletion or 17q gain). A variety of techniques are used for these but they are somewhat more labor intensive.

By performing such molecular tests on hundreds of tumor samples through cooperative group studies, the Children's Oncology Group (COG) [and the former Children's Cancer Group (CCG) and Pediatric Oncology Group (POG)] has been able to shed some light on the relevance of ploidy in neuroblastoma. Neuroblastomas with diploid or tetraploid DNA content are more likely to arise in older children and present with metastatic disease. These same cells are more likely to harbor MYCN gene amplification or chromosome arm 1p deletion or 17q gain, each independently associated with high-risk disease. In contrast, near-triploid DNA content (DNA index near 1.5) is most commonly found in infant tumors that remain localized and are associated with a very favorable

outcome. Thus, ploidy is a surrogate marker that tells us whether certain high-risk genetic features are likely to be present in a neuroblast. Extending this further, the correlation between ploidy and certain genetic aberrations in neuroblastomas has been used to provide a genetic model for different forms of the disease (5). In this model a precursor neuroblast obtains initial genetic alteration either from altered chromosome segregation (called mitotic dysfunction) or from alternative chromosome damage mechanisms. In the former case, cells are predicted to be hyperdiploid with gains of whole chromosomes, whereas in the latter cases they may be diploid or tetraploid with gross chromosomal alterations. For those interested in more detailed treatise on the significance and biological mechanisms of altered ploidy in neuroblastoma, see the recent review by Kaneko and Knudson (6).

In the summary, the ploidy of neuroblastoma cells appears to correlate with tumor behavior likely as a surrogate maker for the presence or absence of specific genetic alterations. For many patients with neuroblastoma, however, determination of age, tumor stage, MYCN status (amplified or not) and histology (favorable or unfavorable) provide enough predictive information to assess tumor risk and choose a treatment or appropriate intensity. The addition of ploidy as a marker adds little additional information except in certain circumstances (particularly in 4S patients and other infants). However, researchers continue to explore the correlation of ploidy with neuroblast behavior.

References:

1.	Green, D.R. and Evan, G. I. A matter of life and death. 1: 19-30, 2002.
2.	Look, A. T., Hayes, F.A., Nitschke, R., McWilliams,

N. B., and Green, A. A. Cellular DNA content as a predictor of response to chemotherapy in infants with unresectable neuroblatoma. New Engl. J. Med., 311: 231-235, 1984.

3.   Look, A. T., Hayes, F. A., Shuster, J. J., Douglass, E. C., Castleberry, R. P., and Brodeur, G. M. Clincal relevance of tumor cell ploidy and N-myc gene amplification in childhood neuroblastoma. A Pediatric Oncology Group Study. J. Clin. Oncol., 9: 581-591, 1991.

4.   Kaneko, Y., Kanda, N., Maseki, N., Sakurai, M., Tsuchida, Y., Takeda, T., Okabe, I., and Sakurai, M. Different karyotypic patterns in early and advanced stage neuroblastomas. Cancer Res., 47: 311-318, 1987.

5.   Brodeur, G. M. Neuroblastoma: biological insights into a clinical enigma. 3: 203-216, 2003.

6.   Kaneko, Y. and Knudson, A. G. Mechanism and relevance of ploidy in neuroblastoma. Genes Chrom Cancer, 29: 89-95, 2000.