

Ras and Seppuku in Neuroblastoma

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Neuroblastoma is a malignant tumor of the peripheral nervous system found almost exclusively in young children (1). More commonly than any other malignancy (2), neuroblastoma can undergo complete spontaneous regression without therapy (3–6). Unfortunately, spontaneous regression of neuroblastomas that present clinically is still a rare phenomenon, but it is frequently observed in stage 4-S tumors (infants who present with localized primary tumors and metastases limited to liver, skin, and/or bone marrow) (1,7,8). Intrinsic tumor cell biology is critical in determining spontaneous regression, because stage 4-S tumors rarely have unfavorable biologic features (such as MYCN gene amplification) (8), while infants with MYCN-amplified tumors (most often stage 4) commonly develop relentless progressive disease in spite of chemotherapy (9).

Spontaneous regression of neuroblastoma may not be limited to stage 4-S tumors and could include some localized (non-stage 4-S) tumors, but surgical resection of clinically detected localized tumors has precluded documentation of spontaneous regression. A high incidence of *in situ* neuroblastomas in patients dying from causes other than tumor (10) and of complete regression of localized tumors treated with only partial resection (11) both suggest that some non-stage 4-S neuroblastomas also can undergo spontaneous regression.

The implementation of nationwide screening for neuroblastoma in Japan (by testing urinary catecholamine metabolites from all infants) has resulted in the detection of many neuroblastomas that presumably would have undergone spontaneous regression, never to have manifested clinically (12). Recent studies using observation instead of therapy for such tumors has confirmed the ability of many limited-stage tumors seen in infants to undergo spontaneous regression (13–15).

The biologic basis by which neuroblastomas undergo spontaneous regression has fascinated pediatric oncologists and tumor biologists for decades. Understanding why it occurs (and why it does not) should provide molecular markers predictive of tumor behavior that will facilitate minimizing therapy for patients destined to cure themselves. At the same time, understanding the mechanisms by which tumors spontaneously regress may point the way toward novel therapies for those patients with high-risk tumors that require very intensive, multimodal therapeutic intervention. Maturation from primitive neuroblast-like malignant cells to well-differentiated and benign tumors (known as ganglioneuromas) has been documented during spontaneous regression of neuroblastomas (6,16,17), and treating high-risk patients with the differentiation inducer 13-*cis*-retinoic acid after myeloablative therapy improves event-free survival (18). However, most spontaneously regressing neuroblastomas do not leave behind well-differentiated tissues. Instead, the tumors simply disappear.

Both cellular (19) and humoral (20) immunologic attack of neuroblastoma cells have been postulated as mechanisms of spontaneous regression, but demonstration that immunologic

effectors are causative in regressing tumors has been elusive. Moreover, the lack of regression in tumors with aggressive biologic features would argue against an immunologic mechanism and suggests a mechanism dependent on tumor cell biology. Hiyama et al. (21) demonstrated both a lack of telomerase activity and shortening of telomeres in stage 4-S neuroblastomas that underwent spontaneous regression, suggesting that tumor proliferation without telomerase eventually triggered tumor senescence in these cells (21,22). Death in cells with critically short telomeres appears to be apoptotic in nature (23) and, while apoptosis can be detected in tumors with favorable biology (24–26), some studies did not detect apoptosis (i.e., internucleosomal DNA fragmentation by terminal deoxynucleotidyl transferase [TdT] labeling) in stage 4-S tumors (27) or in regressing or stable localized tumors detected by mass screening (14). Thus, spontaneous regression of neuroblastoma may occur via more than one mechanism.

Kitanaka and colleagues (28) studied neuroblastomas obtained from patients detected by mass screening in Japan and have compared them to clinically-detected, advanced-stage tumors in patients older than 1 year of age. Prior studies have shown that patients with tumors showing high expression of the proto-oncogene Ras had a favorable outcome relative to patients with tumors showing low Ras expression (29,30). Kitanaka et al. found Ras expression was increased in areas of cellular degeneration, and the latter observation was seen twice as frequently in tumors from case subjects detected in mass screening as in the high-stage tumors. The degenerating cells found in areas of Ras expression did not have the nuclear features, DNA fragmentation, or caspase activation characteristic of apoptosis. Moreover, the authors were able to induce nonapoptotic, caspase-independent cell death in neuroblastoma cell lines by Ras transfection, especially if Ras was transfected together with the neurotrophin receptor Trk A and cells were treated with the Trk A ligand nerve growth factor (NGF). The latter observation is consistent with prior work showing higher Ras (29,30) or Trk A (31) expression in neuroblastomas from patients with a favorable outcome but contrasts with *in vitro* studies showing that Ras promotes survival in cultured sympathetic neurons (32). The effect of Ras and/or Trk A may depend on the nature of the cell in which either (or both) are expressed, and further studies of Ras with biologically diverse neuroblastoma cell lines are warranted.

The paper by Kitanaka et al. joins the increasing body of literature on tumor cell death via caspase-independent mechanisms (33,34). However, in this latest observation, the authors have provided evidence that “spontaneous” programmed cell

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death (PCD) may occur via a caspase-independent, nonapoptotic mechanism. Spontaneous regression of neuroblastoma is thought to be related to those mechanisms used by the vast excess of normal neuroblasts generated during development to graciously exit the scene when no longer needed (35). This latter process has been commonly attributed to “classical” apoptosis and is thought to involve signaling (or the lack of it) via neurotrophins (31,36–38). Because classical apoptosis is well described in neural development (35), and has been detected (although inconsistently) in neuroblastomas that can spontaneously regress (24–26), it is possible that both a caspase-dependent and a caspase-independent form of PCD play a role in regression of neuroblastoma (and perhaps also of developing neuroblasts). Thus, it will be interesting to see if future studies identify this novel mechanism of PCD in normal cells during development.

Kitanaka et al. speculate that Ras expression may contribute a favorable prognosis in nonregressing neuroblastomas treated with chemotherapy. While this is an intriguing idea, it goes against the large body of data showing that currently used chemotherapy (which is highly effective in most favorable-prognosis neuroblastomas) generally acts via apoptosis (39,40). Even poor-prognosis neuroblastomas initially respond well to chemotherapy, only to return later as drug-refractory tumors (41). Prior to therapy, most neuroblastomas lack TP53 mutations, while drug or radiation-resistant neuroblastomas show loss of p53 function (often via mutation). These latter observations point toward a p53-dependent apoptosis as the principle mechanism of cell death in response to current chemotherapy and to loss of p53 function as a major mechanism of drug resistance (41). Drugs that induce both apoptotic and nonapoptotic cell death in neuroblastomas (in a p53-independent manner) are in development (42,43). The relationship of Ras-mediated PCD to chemotherapy-mediated nonapoptotic cell death should be investigated and may provide a further molecular understanding of how to kill tumor cells able to resist “standard” chemotherapy.

Clearly more work is needed to determine if apoptosis, non-apoptotic programmed cell death, or both predominate in spontaneous regression of neuroblastoma, and if the predominant mechanism varies from tumor to tumor. The instigator(s) of neuroblastoma spontaneous regression also deserve further investigation, with telomere shortening being the best documented mechanism to date (21). Kitanaka et al. have not only shed light on a possible new mechanism of cell death, but for neuroblastoma they have also implicated Ras as a player in the process, along with the neurotrophin Trk A and its ligand NGF as possible assistants. In feudal Japan, seppuku (ritual suicide) was an integral part of the bushido (warrior code). The principal, sometimes somberly encouraged by friends, disemboweled himself with a knife (the kozuka) just prior to being beheaded by his “second,” the kaishaku-nin. Whether Ras plays the role of kozuka, kaishaku-nin, or encouraging friend, remains to be elucidated.

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