The Butler Did It: Search for Killer(s) of Kaposi’s Sarcoma Cells in Preparations of Human Chorionic Gonadotropin

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Since its original description more than a century ago, Kaposi’s sarcoma (KS) was considered to be a rare dermatologic condition, and it attracted only limited attention. The association with immune deficiency, first noticed in patients who had undergone renal transplantation, started to generate more interest in this disease. The turning point, however, was the finding of a relationship between KS and acquired immunodeficiency syndrome (AIDS). KS is now recognized as the most prominent tumor of patients with AIDS, particularly common (>30%) among homosexual men. It is not surprising, therefore, that the interest in its etiology, pathogenesis, and therapy is widespread.

Unfortunately, the treatment options for KS are limited. Radiotherapy or chemotherapy as well as the administration of interferon alfa have little impact on the disease. Furthermore, these treatments are toxic, especially when used in combination with antiviral therapies. It is not surprising, therefore, that the finding that sera from pregnant women, as well as commercial preparations of human chorionic gonadotropin (hCG), exhibit in vitro and in vivo activity against KS (1,2) stirred wide interest and raised hope for more efficient treatment. Phase I clinical trials of hCG demonstrated that partial remissions and stabilization of the disease could be achieved in more than half of the patients, with no evidence of toxicity (3). In a study reported in this issue of the Journal, Samaniego et al. (4) show that hCG preparations affect the expression of the apoptosis-modulating proteins c-Myc and c-Rel and induce apoptosis in KS cells in vitro and in vivo. It is interesting that hCG preparations were also shown to induce apoptosis in leukemia cells, as detected in vivo in patients with acute myelogenous leukemia, and to suppress proliferation of HL-60 leukemia cells (5). Reduced expression of the anti-apoptotic protein Bcl-2 was also observed in hCG-treated leukemia cells (5).

The mechanism of hCG’s anti-KS activity is clouded in mystery. Since highly purified or recombinant hCG is inactive, it is presumed that the activity is not associated with hCG itself but with one or more factors that are co-purified with the hormone. These anti-KS factors are present in the urine of pregnant women and mice and are found in different proportions in hCG preparations from different vendors (6–8).

What is the nature of the factors present in hCG preparations that are toxic to KS cells? The available evidence points in two directions. One is focused on low-molecular-weight contaminants. Several observations indicate that the 2–4-kd components exert anti-KS activity (6,8). These components suppress proliferation of KS cells, presumably by reducing expression of AP-1, a complex of transcriptional activators of the immediate-early response genes associated with cell growth (8). It is possible that active factors in hCG represent products of partial degradation of this protein. It has been proposed that the degradation product of the β-hCG subunit (β-core) is conformationally similar to several growth factors (9) may act as an antagonist for growth factor receptors and, therefore, may suppress cell proliferation (8).

The plot, however, is not so simple. Another suspect that may be involved in anti-KS activity is a cytoxic ribonuclease. Griffiths et al. (10) have isolated an 18-kd ribonuclease (RNase) from hCG preparations and have demonstrated its strong cytotoxic activity against KS cells. During purification, the ribonuclease was closely associated with the β-core subunit of hCG. The authors suggest that the overall anti-KS activity of hCG might result from the combined effects of fragmented hCG and RNase activity (10). The nonsecretory 34-kd RNase, which after deglycosylation remains enzymatically active and fits the size (18 kD) of the enzyme isolated by Griffiths et al. (10), was detected in the urine of pregnant women (11,12). This enzyme is homologous to an eosinophil-derived neurotoxin (EDN), one of two RNase A-superfamily members found in granules of human eosinophils (13). The other family member is eosinophil cationic protein (ECP). The function of these RNases is controversial, but both are cytotoxic, particularly to parasites. Amino terminal-deglycosylated recombinant human ECP has been found to have direct antiviral activity against a single-stranded RNA virus, respiratory syncytial virus group B (13).

Additional evidence implicating RNase activity as the culprit responsible for the anti-KS effects of hCG comes from the recent observation of Newton and Rybak (14). These authors have reported that human EDN (hEDN), genetically engineered by appending to the amino-terminus of hEDN four amino acid residues corresponding to the proximal part of the hEDN signal peptide (serine, leucine, histidine, and valine, at the -4 to -1 positions, respectively), was cytotoxic to KS cells but had little effect on four other tumor cell lines (14). This modification of hEDN, designed to resemble the sequence of the hEDN found in the urine of pregnant women (11,12), appears to target this protein to KS cells (14).

Could the characteristic features of the victim, i.e., the nature of changes in target cells exposed to hCG, provide clues regarding the suspect? The fragments of hCG acting as growth factor antagonists are expected to suppress cell growth, perhaps by preventing phosphorylation of the retinoblastoma protein (pRB), which may lead to arrest in the cycle and late apoptosis. The cytoxic RNase, on the other hand, is expected to degrade intracellular RNA and induce cytotoxicity by non-specific inhibition of protein synthesis (15). Here again, the reality is not so simple. Onconase, an amphibian 12-kd RNase (16) that is cytotoxic to tumor cells (17) and is in phase III clinical trials (18) was recently shown to suppress cell cycle progression of U-937...
human histiocytic lymphoma cells by suppressing expression of cyclin D; increasing abundance of the cyclin-dependent kinase inhibitors (CKIs), p16^INK4a, p21^WAF1/CIP1, and p27^KIP1, and inhibiting phosphorylation of pRB (19). The increase in CKIs was explained as possibly resulting from onconase’s effect via double-stranded RNA-dependent protein kinase (PKR), the enzyme that phosphorylates the protein IkB and thereby activates the ubiquitous transcription factor NFκB (20). Among a multitude of genes activated by NFκB, some regulate cell growth and affect the cell’s propensity to undergo apoptosis. Similar to the apoptotic mode of KS cell death following exposure to hCG (4), onconase also induces apoptosis in target cells (19,21). Thus, the changes in KS cells induced by a cytotoxic RNase may not be easily distinguished from those caused by growth factor antagonists. Conceivably, reduced expression of cyclin D, as observed in the case of onconase-treated U-937 cells (19), might be particularly effective in the suppression of KS cell proliferation, which may be driven by overexpression of human herpesvirus 8-encoded v-cyclin D (22), a homologue of the cellular cyclin D2 gene product. It should be noted, however, that the KS cell line used in the studies by Griffiths et al. (10) and Newton and Rybak (14), i.e., KSY-1, lacks human herpesvirus 8 sequences.

The observations that the sequence modification of RNases (14,23) or their use as immunotoxins (24) enhances cytotoxicity toward particular tumor cell types, including KS (14), has opened new avenues to increase the effectiveness of their therapeutic use. Thus, RNases may become a new class of antitumor agents with clinical utility (23–25). If a cytotoxic RNase indeed plays a major role in the apparent anti-KS activity of hCG, the field is open to further enhance its effectiveness by chemical or genetic engineering. Furthermore, by analogy to findings with onconase [e.g. (21)], the anti-KS RNase may also enhance the cytotoxic effects of other antitumor agents. Such drug combinations may be more effective against KS than the RNase alone.

It may well be that the observed anti-KS activity of hCG preparations results from the effects of both components, i.e., degraded fragments of the hormone (or other low-molecular-weight contaminants, including fragmented hEDN) and hEDN (10,14). The apparent controversy may be due to the fact that these components are present in varying proportions in different hCG preparations. The effect induced by one component, thus, may be predominant over another, when studied in different laboratories. It should be noted, however, that Samaniego et al. (4) do mention that their active hCG preparations had no RNase activity.

Identification and purification of the anti-KS factors contaminating hCG preparations is essential for further progress in their clinical application. Also important, especially if used in combination with other drugs, is understanding the mechanism by which they induce cytostatic and cytotoxic effects. Further studies along these lines, therefore, are needed, to ascertain fully the possibilities of their application in the clinic. Revealing the identity of the real killer of KS cells, rather than suspecting the butler, is certainly the first priority.

REFERENCES


