The cancer-specific expression profile of S-3B prompted us to focus on its effects on tumorigenesis. Normally, the immune system is programmed to eliminate abnormal cells that may become malignant. The elimination of such pre-cancerous cells is mainly attributable to CD8+ T lymphocytes and natural killer (NK) cells. Only when neoplastic cells resist the cytotoxic activity of immune effectors, they can proliferate unrestrained and generate tumors, a process that is known as immune escape. In the course of tumor progression, immune cells of multiple types infiltrate malignant lesions. Some of these cells exert tumor-supporting functions, while others—including NK cells—mediate robust antitumor effects.5

To get insights into the functions of S-3B, we first tested the effect of its overexpression on a non-tumorigenic cell line. We observed that inoculation of originally non-tumorigenic cells that had been engineered to overexpress S-3B in nude mice, which have no T lymphocytes but harbor high NK-cell activity, promoted the development of malignant lesions. We therefore hypothesized that S-3B could inhibit the antitumor activity of NK cells. To test this hypothesis, we downregulated S-3B in vivo by injecting specific small-interfering RNAs (siRNAs) into neoplastic lesions generated by a highly tumorigenic cell line, an intervention that considerably reduced tumor growth. The impact of S-3B on the cytotoxic activity of NK cells was confirmed as the effects of S-3B-targeting siRNAs completely disappeared in NK cell-depleted nude mice. In vitro, S-3B was shown to interact with pro-caspase-8, hence preventing its proteolytic maturation upon the interaction of FAS with its ligand (FASL). S-3B also inhibits the activation of caspase-6, thus increasing the resistance of neoplastic cells to granzyme B and various chemotherapeutics.
of caspases-9, -3, -6 and -7. Since intrinsic apoptosis is also the cell death subroutine whereby most anticancer agents exert their activity, we decided to explore the effects of S-3B on the response of neoplastic cells to staurosporine and 5-fluorouracil. Upon exposure to these treatments, cells expressing high levels of S-3B not only failed to die, but also were able to divide and form colonies in clonogenic assays. In contrast, cells lacking S-3B died massively in response to apoptotic stimuli. By studying the mechanisms underlying intrinsic apoptosis as triggered in our model by staurosporine and 5-fluorouracil, we observed that cancer cells underwent mitochondrial depolarization followed by the activation of caspases-9 and -3 irrespective of S-3B expression levels. Rather, S-3B was involved in the events downstream of caspase-3 activation, notably as it inhibited the cleavage and activation of pro-caspase-6. Thus, S-3B sequestered pro-caspase-6 upon physical interaction, thus impeding its activation by active caspase-3.

Taken together, these data suggest that combining S-3B-targeting interventions with chemotherapy could result in a superior efficacy by improving both the activity of immune cells and the direct cytotoxicity of antineoplastic agents. In support of this hypothesis, we demonstrated that the association of S-3B-depleting siRNAs and 5-fluorouracil improves anticancer responses in mice.

As S-3B appeared to provide cancer cells with improved protection as compared with survivin, through different mechanisms, we studied in detail the sequence of S-3B. S-3B shares the first 113 amino acids with survivin but contains 7 amino acids at the C-terminus that differ from the corresponding residues of survivin. We showed that this short polypeptide, which we named LEO sequence, is required for the interaction of S-3B with its targets.

In conclusion, S-3B turned out to play a critical role in cancer initiation, progression and dissemination (Fig. 1). This protein is not the sole responsible for oncogenesis, but some evidence indicates that the expression level of S-3B might be related to tumor progression1 and poor disease outcome.6,7 The importance of S-3B in the resistance of cancer cells to immune effectors (especially NK cells) and chemotherapy, coupled to its tumor-specific expression pattern, should encourage the development of S-3B-targeting therapies, for example siRNA-based approaches or inhibitors of the LEO domain.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
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