Burning up TNF toxicity for cancer therapy

The tumor-killing capacity and the systemic toxicity of the cytokine tumor necrosis factor (TNF) have appeared inseparable. Now a study shows that TNF loses its toxicity but still kills tumors in heat-treated mice.

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Over a century ago, Coley described an endogenous bacterially induced tumor-killing activity in humans[1]. The responsible mediator, tumor necrosis factor (TNF), was finally isolated in 1975 based on this tumor-killing activity[2]. With the determination of its sequence ten years later, it became clear that TNF was identical to a previously characterized molecule called cachectin, known as a key mediator of shock, wasting and inflammation[3]. This finding dampened the hopes for a general application of TNF in tumor therapy. Clinically, the only success has occurred with isolated limb perfusion for a limited subset of susceptible tumors, such as melanoma and sarcoma[4]. Since then, the big question tantalizing the biomedical community has been how to separate the negative from the positive effects of TNF. A new study by Van Molle *et al.*[5] shows that placing mice into a humid 42 °C oven for 20 min changes the systemic reaction to TNF. Like a filter, this treatment allows the antineoplastic effects to act while blocking the systemic toxicity of TNF.

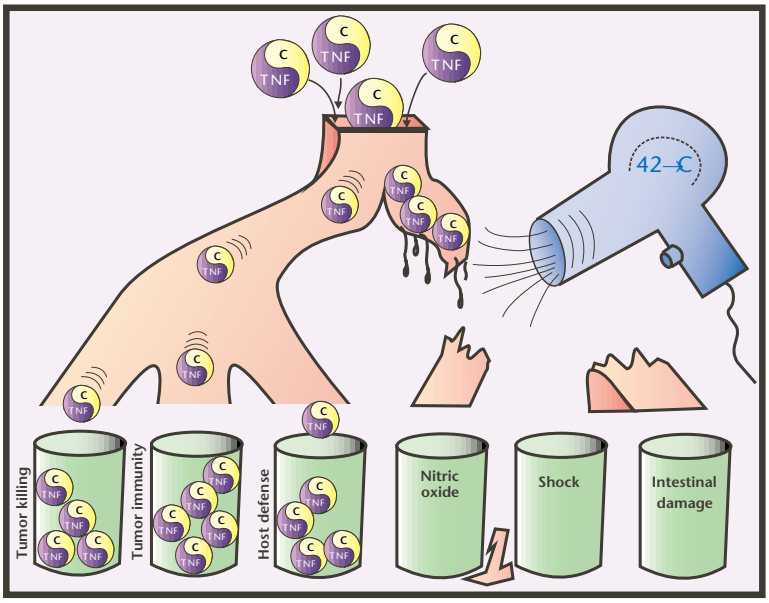

Fig. 1 The TNF slot machine and heat shock: TNF was initially identified on the basis of its tumor-killing activity. But it was revealed later to be identical to a molecule called cachectin (C), involved in shock, wasting and inflammation. Under normal conditions, TNF/cachectin mediates a large variety of responses. These involve, on the one hand, tumor-killing activities and on the other, overshooting cytokine responses, NO overproduction and lethal shock. Under conditions of heat shock the spectrum of the TNF/cachectin response is partially blocked, still allowing tumor elimination, but blocking the toxic side effects on healthy tissue.

What is the molecular basis of this 'Turkish bath' effect? The heat-shock (HS) response is a stress response conserved throughout evolution from prokaryotes to humans[6]. It rallies heat-sensitive transcription factors to launch a coordinated response after transient, sub-lethal heat. The result is reduced expression of most cellular genes and a massive upregulation of a family of chaperones called heat-shock proteins (HSPs). These proteins stabilize misfolded proteins and aid their refolding or elimination. Accordingly, HSPs or heat preconditioning can rescue cells from death induced by denaturing stresses such as prolonged heat exposure or neurodegenerative conditions associated with misfolded proteins[6]. Moreover, certain members of the HSP family appear to have specific functions in the control of cell death. For instance, overexpression of HSP70 in tumor cell lines blocks TNF-induced tumor-cell death, and depletion of endogenous HSP70 increases the sensitivity of tumor-cell cultures to TNF (ref. 7).

The HS response is universal in that it is not only induced by heat, but also by a number of other stressors, such as ischemia, certain metal ions and ethanol. One particular stress seems to be the tumorigenic environment or neoplastic transformation itself. Accordingly, most human carcinomas show elevated HSP70 levels, and further induction of HSP70 does not bring about enhanced survival potential. Thus, tumors have already played the HSP70 joker and used it for securing their basic survival, whereas stress-induced upregulation of this protein in normal cells renders them more resistant to TNF (ref. 6).

HS or overexpression of HSPs not only protects cultured cells, but also has profound protective effects *in vivo*. Examples from animal studies include ischemic conditions of brain and heart, such as occurs during stroke, as well as lethal shock triggered by bacterial endotoxins[6]. Building on this knowledge, van Molle *et al.* challenged mice with whole-body hyperthermia 12 hours before systemic TNF infusion. 75% of the mice survived this otherwise lethal TNF treatment. A number of surrogate markers of the systemic shock response, such as nitric oxide production, hypothermia and intestinal tissue damage were inhibited, correlating with the reduced lethality. Using this model system, the authors then set out to test the role of HSP70 in the preconditioning response (Fig. 1).

Heat treatment failed to protect against TNF-induced lethality in mice deficient for HSP70.1, the major stress-inducible member of the HSP70 family. The next step, geared towards applicability, was to see whether HS was also effective when given repeatedly over long time periods. Daily HS treatment over ten days was effective and even improved the protection from systemic TNF toxicity as compared with a single HS treatment. After the establishment of this experimental system, it was possible to test the feasibility of HS as a protective cotreatment in cytokine-based cancer therapy. Mice inoculated with syngenic melanoma cells were treated for 10 days with high doses of TNF and interferon-δ with or without daily heat preconditioning. Tumors were effec-

tively eliminated in both groups, indicating that HS did not blunt the anticancer effect of TNF. However, while only 10% of the tumor-bearing control mice survived the cytokine therapy, 70% of the mice receiving the combination therapy with HS and cytokines survived both the treatment and the tumor challenge.

Are these findings directly transferable to humans? In the clinic, the overall effectiveness of therapy would be benchmarked against the survival rate of untreated patients. In the given study, 50% of the untreated mice survived their tumor. From this perspective, the treatment benefit would be hardly significant. Moreover, a HS response in humans is infinitely more difficult to induce than in mice. A sauna visit, even at over 100 °C ambient temperature, is definitely not sufficient for a systemic HS response in humans.

The potential use of HS to enable TNF-tumor therapy without deleterious side effects gives hope and opens doors for further research. However, it also raises many questions. The most burning ones are linked to the fact that neither the crucial targets of systemic TNF toxicity nor the mechanism or location of HSP70 protection are fully clarified. In the absence of this mechanistic information extrapolation of protective effects from mouse to humans remains impossible.

The second set of questions concerns the mechanism of tumor killing: why does HS not protect the tumor from TNF? Although TNF can kill many tumor cell lines *in vitro* by direct signaling of apoptosis[2], its effectiveness as an anticancer agent in human patients may occur through a different mechanism: TNF may disrupt the tumor vasculature due to the inactivation of integrin $\perp_{v\_3}$ in endothelial cells[8,9]. The preservation of TNF's tumor-killing ability after the HS may be due to the inability of HSPs to inhibit TNF-induced signaling pathways leading to the inactivation of integrin. However, we do not know how well the murine melanoma xenograft model correlates with human neoplasia, in particular with respect to the role of the vasculature or interactions of the tumor with cells of the immune system.

If the tumor elimination mechanisms differ from mouse to human, then induction of HSP70 might have different or even opposite effects in people. In the worst case, protective HSP70 might be induced in tumors that have low HSP70 expression. However, HSP70 induction in tumors need not necessarily discourage such approaches. On the contrary, HSP70 may enhance the elimination of tumors by the immune system. In tumor cells, HSP70 can be displayed on the cell surface as a recognition marker for natural killer cells[10], and it can function in presenting tumor-specific peptides to the immune system, enabling tumor recognition and elimination[11]. Thus, the overall outcome of HS is hardly predictable and full understanding still requires a great deal of experimentation.

Once these outstanding questions have been solved, HS preconditioning might ultimately turn out to be a valuable addition to the oncologists' choices for therapy refinement. This requires establishment of protocols to achieve a powerful HS response, possibly not with heat—but rather with other stressors like zinc or tin ions. Another possibility for the future might be a reverse strategy. Many tumors are protected by their relatively high HSP70 content, and strategies for the reduction of HSP70 in tumor cells enhance spontaneous tumor cell death *in vitro* as well as susceptibility to TNF (refs. 7,12). The proof of concept of tumor elimination based on reduced HSP70 must come from *in vivo* experiments. The results could underline the concept that the ratio of HSP70 expression in tumors and normal cells might determine the systemic side effects of antineoplastic therapy with TNF.

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