Figure 2 Morphology of microinjected NRK cells. **a**, Control cells. **b**, Wild-type cells injected with 20 μM cytochrome *c*. **c**, Wild type cells preincubated with Z-VAD-fmk for 30 min and co-injected with 20 μM cytochrome *c* and 0.1 μg μl^{-1} TRITC. **d**, Fluorescence micrograph of the same field as that in (c). **e**, Cells over-expressing Bcl-2 co-injected with 20 μM cytochrome *c* and 0.1 μg μl^{-1} TRITC. **f**, Fluorescence micrograph of the same field as that in (e).

and Bcl-2 overexpression, respectively (Fig. 2d, f).

Our results indicate that injected cytochrome *c* can induce apoptosis in various cell types, and that this effect is caspase-dependent. It has been suggested that the role of Bcl-2 protection is restricted to its mitochondrial location, where it prevents leakage of cytochrome *c*[5,6]. Microinjection of cytochrome *c* bypasses the need for release of this protein from mitochondria. However, cells overexpressing Bcl-2 were also protected from the apoptosis induced by injecting cytochrome *c*. This could be explained in several ways.

First, Bcl-2 is not restricted exclusively to the mitochondrial membrane, hence some of its anti-apoptotic property might be based outside this location[1]. Second, Bcl-2 may be a member of the 'apoptosome' complex and it could prevent cell death by direct binding to Apaf-1/Apaf-2/Apaf-3, in a similar way to CED-9's activity on CED-3/CED-4 proteins[9]. Third, it has been reported that cytochrome *c* binds to Bcl-X$_L$ (Ref. 10). Bcl-2 may exert a similar quenching effect outside mitochondria. Fourth, Bcl-2 targets several proteins, including

Raf-1, to mitochondria[11]. Therefore when overexpressed, Bcl-2 could possibly act as a transporter for cytochrome *c* back into mitochondria[12].

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Tumour regression after endostatin therapy

It was with great interest that we read the report[1] on the lack of acquired drug resistance to repeated doses of endostatin in experimental cancers in mice. Of particular interest is the unprecedented finding that each tumour type became indefinitely dormant after a varying number of treatment cycles. We believe one explanation for this phenomenon might be found in the clinical observation that, after complete regression of large bulky tumours, rebiopsy of the primary tumour site will frequently show large amounts of fibrosis or scarring.

Pharmacological doses of endostatin, a C-terminal fragment of collagen XVIII with relative molecular mass 20,000, at very low concentrations may be deposited in the extracellular matrix with each cycle of therapy, analogous to amyloid deposits in patients with light-chain disease. The marked shrinkage of the mouse tumours from approximately 250–450 mm^3 to 5–50 mm^3 could effectively increase the local extracellular matrix concentration of endostatin 5–50 fold. Each successive cycle of regrowth followed by treatment and regression could progressively increase the concentration of endostatin in the local extracellular matrix until the concentration is sufficient to inhibit further angiogenesis.

This phenomenon of preferential concentration could explain the observation of a dormant primary tumour while the same tumour type inoculated at a distant site would be uninhibited. The extracellular matrix concentration of endostatin at these distant sites could be several log factors below the concentration at the primary tumour site and insufficient to inhibit tumour-driven angiogenesis.

If this hypothesis is correct, it could mean Boehm *et al.* have serendipitously discovered the ideal way to administer endostatin therapy, delivering a high inhibitory concentration of endostatin at the primary tumour site while keeping the systemic extracellular matrix concentration below the level necessary to inhibit naturally occurring angiogenesis such as wound healing. An assay of the relative concentration of endostatin at the primary tumour site compared to distant tissue concentration should establish if this is the basis for the observed phenomenon.

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