The Role of Cytokines in Cancer Cachexia

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Introduction

The cachectic syndrome, characterised by marked weight loss, anorexia, asthaenia, and anaemia, is invariably associated with the presence and growth of the tumour and leads to a malnutrition status due to the induction of anorexia or decreased food intake. In addition, the competition for nutrients between the tumour and the host leads to an accelerated starvation state that promotes severe metabolic disturbances in the host, including hypermetabolism, which leads to decreased energetic efficiency. Although the search for the cachectic factor(s) started a long time ago, and although many scientific and economic efforts have been devoted to its discovery, we are still far from a complete understanding of cancer cachexia. The chapter discusses the different signalling pathways, particularly the role of transcriptional factors, involved in muscle wasting. The main aim is to summarise and evaluate the different molecular mechanisms and catabolic mediators (both humoral and tumoural) involved in cancer cachexia, since they may represent targets for promising future clinical investigations.

Cytokines

Cytokines have a key role as the main humoural factors involved in cancer cachexia (Fig. 1), and a large number of them may be responsible for the metabolic changes associated with cancer wasting.

Anorexia may account for malnutrition, invari-

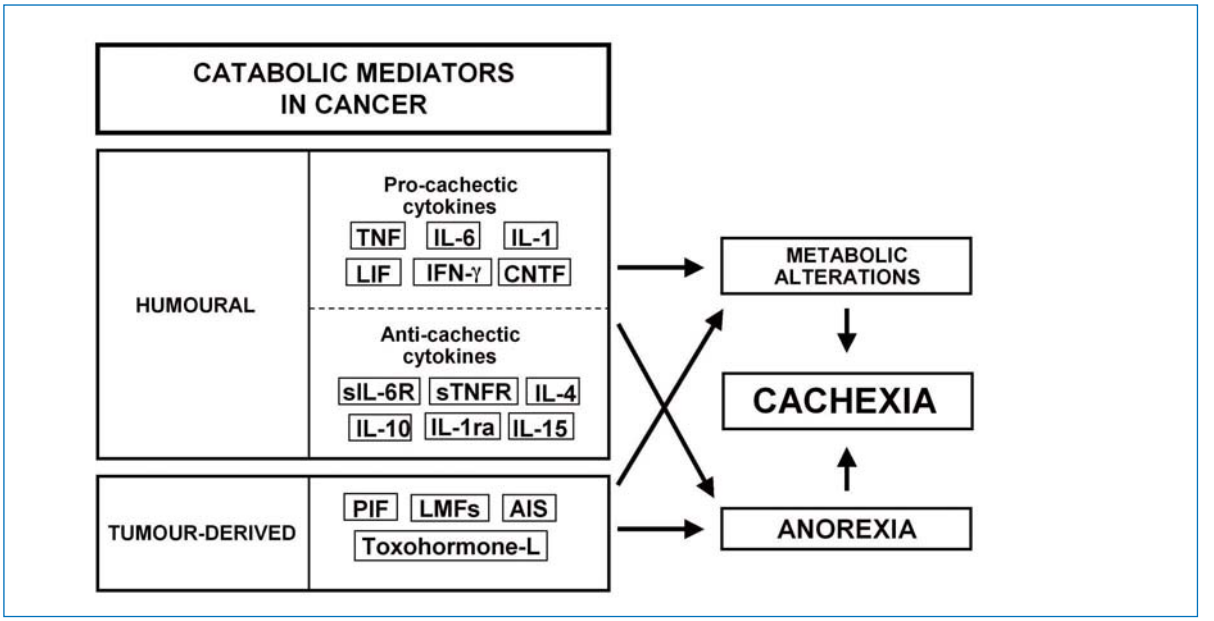

Fig. 1. Catabolic mediators in cancer. Both tumour-derived and humoural (cytokines) factors are involved in mediating the anorexia and metabolic changes characteristic of the cachectic state. For abbreviations, see text

ably associated with cancer cachexia; but, are cytokines involved in the induction of anorexia? Cytokines, such as interleukin (IL)-1 and tumour necrosis factor (TNF)-α have been suggested to be involved in cancer-related anorexia, possibly by increasing the levels of corticotropin-releasing hormone (CRH), a central nervous system neurotransmitter that suppresses food intake, and the firing of glucose-sensitive neurons, which would also decrease food intake. However, many other mediators may be involved in cancer-induced anorexia. Leptin (an adiposity signal to the hypothalamus that it is a member of the cytokine family) does not seem to play a role, at least in experimental models [1, 2], and in human subjects, cancer anorexia does not seem to be due to a dysregulation of leptin production [3]. Indeed, leptin concentrations are not elevated in weight-losing cancer patients [4, 5] and are inversely related to the intensity of the inflammatory response [6] and the levels of inflammatory cytokines [7, 8]. Concentrations of the peptide seem to be dependent only on the total amount of adipose tissue present in the patient. Cytokines have been implicated in cancer-induced anorexia since they modulate gastric motility and emptying, either directly in the gastrointestinal system or via the brain, by altering efferent signals that regulate satiety. IL-1, in particular, has been clearly associated with the induction of anorexia [9] in that it blocks neuropeptide Y (NPY)-induced feeding. The levels of this molecule (a feeding-stimulating peptide) are reduced in anorectic tumour-bearing rats [10], and a correlation between food intake and brain-IL-1 has been found in anorectic rats with cancer. The mechanism involved in the attenuation of NPY activity by cytokines may be related to an inhibition of cell firing rates or to an inhibition of NPY synthesis or an attenuation of its postsynaptic effects [11]. Other mediators have been proposed [12], including changes in the circulating levels of free tryptophan; these may induce changes in serotonin brain concentrations and, consequently, cause changes in food intake. Bing et al. [13] suggested that some tumour-derived compounds may mediate anorexia associated with tumour burden.

Different experimental approaches have demonstrated that cytokines are able to induce weight loss. Nevertheless, the results obtained have to be carefully interpreted. Thus, episodic TNF-α administration has proved unsuccessful at inducing cachexia in experimental animals. Indeed, repetitive TNF-α administrations initially induce a cachectic effect, but tolerance to the cytokine soon develops and food intake and body weight return to normal. Other studies have shown that escalating doses of TNF-α are necessary to maintain the cachectic effects.

Strassman et al. [14] have shown that treatment with an anti-mouse IL-6 antibody reversed the key parameters of cachexia in murine colon adenocarcinoma tumour-bearing mice. These results seem to indicate that, at least in certain types of tumours, IL-6 has a more direct involvement than TNF-α in the cachectic state. Similar results were obtained in a mouse model that reproduced the cachexia associated with multiple myeloma [15, 16] and in a murine model of intracerebral injection of human tumours [17]. Conversely, other studies have shown in a very similar mouse tumour model that IL-6 is not involved in cachexia, and studies using incubated rat skeletal muscle have clearly demonstrated that IL-6 had no direct effect on muscle proteolysis.

Another interesting candidate for cachexia is interferon (IFN)-γ, which is produced by activated T and NK cells and possesses biological activities that overlap with those of TNF-α. Matthys et al. [18] used a monoclonal antibody against IFN-γ to reverse the wasting syndrome associated with growth of the Lewis lung carcinoma in mice, thus indicating that endogenous production of IFN-γ occurs in the tumour-bearing mice and is instrumental in bringing about some of the metabolic changes characteristic of cancer cachexia. The same group also demonstrated that severe cachexia develops rapidly in nude mice inoculated with CHO cells constitutively producing IFN-γ as a result of the transfection of the corresponding gene.

Other cytokines, such as leukaemia inhibitory factor (LIF), transforming growth factor (TGF)-β, or IL-1 have also been suggested as mediators of cachexia. Thus, mice engrafted with tumours secreting LIF developed severe cachexia. Concerning IL-1, although its anorectic and pyrogenic effects are well-known, administration of IL-

1 receptor antagonist (IL-1ra) to tumour-bearing rats did not result in any improvement in the degree of cachexia, so that the role of this cytokine in cancer cachexia may be secondary to the actions of other mediators. Interestingly, the levels of both IL-6 and LIF are increased in patients with different types of malignancies.

Ciliary neurotrophic factor (CNTF) is a member of the family of cytokines that includes IL-6 and LIF, and is produced predominantly by glial cells of the peripheral nervous system; however, this cytokine also seems to be expressed in skeletal muscle. Henderson et al. [19] demonstrated that CNTF induces potent cachectic effects and the production of acute-phase proteins (independent of the induction of other cytokine family members) in mice implanted with C6 glioma cells, genetically modified to secrete this cytokine. CNTF, however, exerted divergent direct effects dependent on the dose and exposure time of in vitro muscle preparations [20].

If anorexia is not the only factor involved in cancer cachexia, it becomes clear that metabolic abnormalities leading to a hypermetabolic state must have a very important role. Interestingly, injection of low doses of TNF-α, either peripherally or into the brain of laboratory animals, elicits rapid increases in the metabolic rate that are not associated with increased metabolic activity but rather with an increase in blood flow and thermogenic activity of brown adipose tissue (BAT), associated with uncoupling protein-1 (UCP1). During cachectic states, there is an increase in BAT thermogenesis, both in humans and experimental animals. Until recently, UCP1 (present only in BAT) was considered to be the only mitochondrial protein carrier that stimulated heat production, by dissipating the proton gradient generated during respiration across the inner mitochondrial membrane and therefore uncoupling respiration from ATP synthesis. However, two additional proteins sharing the same function, UCP2 and UCP3, have since been described. While UCP2 is expressed ubiquitously, UCP3 is expressed abundantly and specifically in skeletal muscle in humans and in BAT of rodents. Our research group has demonstrated that both UCP2 and UCP3 mRNAs are elevated in skeletal muscle during tumour growth and that the effect of TNF-α mimics the increase in gene expression induced by these proteins [21]. In addition, TNF-α induces uncoupling of mitochondrial respiration, as recently shown in isolated mitochondria [22].

Several cytokines have been shown to mimic many of the metabolic abnormalities found in the cancer patient during cachexia. Among these metabolic disturbances, changes in lipid metabolism, skeletal muscle proteolysis and apoptosis, and acute-phase protein synthesis have been described [23]. Concerning muscle wasting, it seems that administration of TNF-α to rats results in increased proteolysis of skeletal muscle, associated with an increase in gene expression and higher levels of free and conjugated ubiquitin, both in experimental animals [24] and humans [25]. In addition, the in vivo action of TNF-α during cancer cachexia does not seem to be mediated by IL-1 or glucocorticoids. Other cytokines, such as IL-1 or IFN-γ, also activate ubiquitin gene expression. Therefore, TNF-α, alone or in combination with other cytokines [26], seems to mediate most of the changes concerning nitrogen metabolism associated with cachectic states. In addition to the massive muscle protein loss, and similar to that observed in skeletal muscle of patients with chronic heart failure who also suffer from cardiac cachexia [27], muscle DNA is also decreased during cancer cachexia, leading to DNA fragmentation and apoptosis [28, 29]. Moreover, TNF-α can mimic the apoptotic response in muscle of healthy animals [30].

Factors Other Than Cytokines

In addition to humoural factors, tumour-derived molecules have also been suggested as mediators of cancer cachexia. Firstly, cancer cells are capable of constitutively producing cytokines. These may act on cancer cells in an autocrine manner or on supporting tissues, such as fibroblasts and blood vessels, to produce an environment conducive to cancer growth [31]. While tumour-produced cytokines may have a more important role in the anorexia-cachexia syndrome, several compounds produced by the host [32] are likely to have an important role in mimicking the metabolic

changes associated with the cachectic state.

Perhaps the first evidence of tumour-derived catabolic factors came from studies with Krebs-2 carcinoma cells in mice; inactive extracts of these cells induced cachexia when injected into normal non-tumour-bearing mice [33]. Similarly, Kitada et al. [34] purified a low-molecular-mass (< 10 kDa) proteinaceous material from extract of thymic lymphoma in AKR mice that showed lipolytic activity in rat adipocyte suspensions. Thus, extracts of thymic lymphoma, conditioned medium from thymic lymphoma cell lines, and serum from lymphoma-bearing mice cause lipid mobilisation in experimental animals. Toxohormone L, a polypeptide of approximately 75 kDa, was isolated from the ascites fluid of hepatoma patients and sarcoma-bearing mice; it induces lipid mobilisation, immunosuppression, and involution of the thymus [35].

Tisdale's group at the University of Aston (UK) described and characterised a lipid-mobilising factor (LMF) that induces lipolysis in adipose tissue, in association with stimulation of adenylate cyclase activity [36]. Although this factor was originally purified from a cachexia-inducing mouse colon adenocarcinoma (MAC16). It has also been found in the urine of cancer patients, suggesting that it can induce lipid mobilisation and catabolism in cachectic cancer patients [37]. In fact, LMF is homologous to the plasma protease Zn-α2-glycoprotein (ZAG) in amino-acid sequence, electrophoretic mobility, and immunoreactivity. The 2.8 Å crystal structure of ZAG resembles a class I major histocompatibility complex (MHC) heavy chain, although it does not bind class I light chain β2-microglobulin. The ZAG structure includes a large groove analogous to class I MHC peptide binding grooves. Instead of a peptide, the ZAG groove contains a nonpeptidic compound implicated in lipid metabolism under pathological conditions. Hirai et al. [38] also suggested that LMF has a role in initiating hepatic glycogenolysis during experimental cancer cachexia through an increase in cyclic AMP in liver.

Anaemia-inducing factor (AIS), an approximately 50-kDa protein secreted by malignant tumour tissue, depresses erythrocyte and immunocompetent cell functions. AIS reduces food intake, body weight, and body fat in rabbits; it also shows an important lipolytic activity [39].

Todorov et al. [40] purified and characterised a 24-kDa proteoglycan, present in experimental animals [41] and in the urine of cachectic patients [42], that seems to account for increased muscle protein degradation and decreased protein synthesis [43]. This compound, known as PIF (proteolysis -inducing factor), activates protein degradation specifically through stimulation of the ATP-proteasome-dependent pathway. Injection of the compound into healthy animals results in muscle wasting, similar to that associated with experimental cancer cachexia. In vitro studies on C2C12 myoblasts have shown that eicosapentaenoic acid (EPA) blocks PIF action on proteolysis, in addition to suggesting that PIF acts intracellularly via the arachidonate metabolite 15-hydroxyeicosatetraenoic acid (15-HETE) [44]. PIF also increases NFκB expression in cultured cells (M. Tisdale, personal communication). Therefore, PIF may have a constitutive role in normal states and become altered or overproduced during cancer cachexia, with important effects on muscle protein catabolism and acute-phase protein (APP) synthesis in this pathological state (Fig. 2).

Transcriptional Factors

At the moment there are few studies describing the involvement of different transcriptional factors in muscle wasting. Penner et al. [45] reported an increase in NFκB and AP-1 transcription factors during sepsis in experimental animals. Recent data from our laboratory do not support an involvement of NFκB in skeletal muscle during cancer cachexia (unpublished data). However, tumour burden results in a significant increase in the binding activity of AP-1. Interestingly, inhibition of NFκB is not able to revert muscle wasting in cachectic tumour-bearing animals [46]; however, inhibition of AP-1 results in a partial reversal of protein degradation in skeletal muscle associated with tumour growth (unpublished data). The increase in NFκB observed in skeletal muscle during sepsis can be mimicked by TNF-α. Indeed, TNF-α addition to C2C12 muscle cultures results

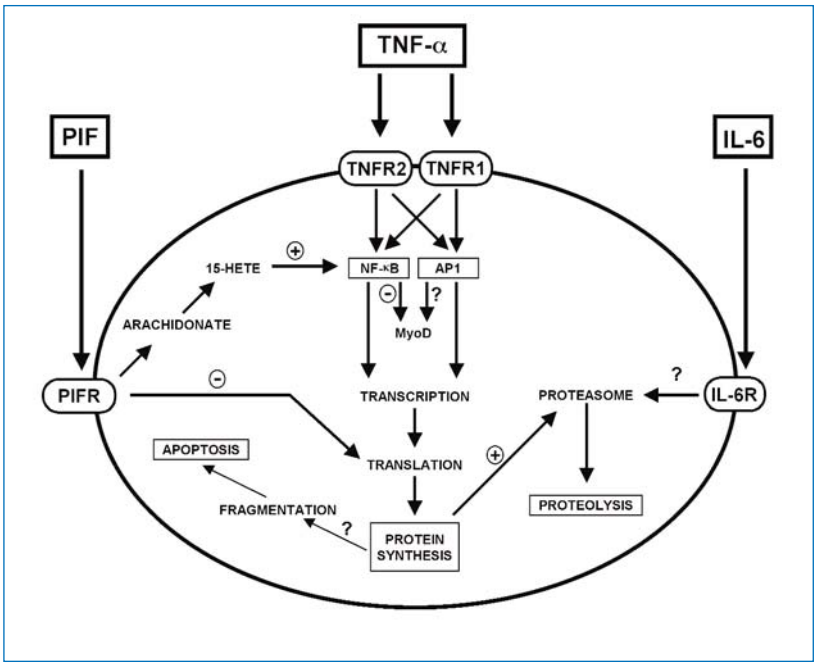

Fig. 2. Interactions between pro-inflammatory cytokines and PIF. Both humoural (TNF-α and IL-6) and tumoural (PIF) factors have been shown to activate intracellular muscle proteolysis by different mechanisms, but possibly sharing common pathways. For abbreviations, see text

in a short-term increase in NFκB [47, 48]; however, whether or not this increase in NFκB promoted by TNF-α is associated with increased proteolysis and/or increased apoptosis in skeletal muscle remains to be established. In relation to AP-1 activation, TNF-α has been shown to increase c-jun expression in C2C12 cells [49]; in turn, the effects of c-jun overexpression mimic those of TNF-α on differentiation, i.e. decreased myoblast differentiation [50]. Tumour mediators, PIF in particular, also seem to be able to increase NFκB expression in cultured muscle cells, this possibly being linked with increased proteolysis (M. Tisdale, personal communication). Other transcriptional factors reported to be involved in muscle changes associated with catabolic conditions include c/EBPβ and c/EBPδ, which are increased in skeletal muscle during sepsis [51], PW-1, and PGC-1. TNF-α decreases MyoD content in cultured myoblasts [52] and blocks differentiation by a mechanism that seems to be independent of NFκB and that involves PW-1, a transcriptional factor related to p53-induced apoptosis [53]. Cytokine action on muscle cells, therefore, seems to rely most likely on satellite cells blocking muscle differentiation or, in other words, regeneration.

Finally, the transcription factor PGC-1 has been associated with the activation of UCP2 and UCP3 and with increased oxygen consumption by cytokines in cultured myotubes [54]. This transcriptional factor is involved as an activator of PPAR-γ in the expression of uncoupling proteins.

Strategies To Fight Cachexia Based on Cytokines and Transcriptional Factors

Since both anorexia and metabolic disturbances are involved in cancer cachexia, the development of different therapeutic strategies has focused on these two factors. Unfortunately, counteracting anorexia either pharmacologically or nutritionally has led to rather disappointing results in the treatment of cancer cachexia. It is basically for this reason that the strategies mentioned below rely on neutralising the metabolic changes induced by the tumour, which are ultimately responsible for the weight loss. Therefore, taking into account the involvement of cytokines in cachexia, therapeutic strategies have been aimed at blocking either their synthesis or their action.

For instance, TNF-α synthesis inhibitors have been used therapeutically. Pentoxifylline, a methylx-

antine derivative, was originally employed for the treatment of various types of vascular insufficiency, because of its haemorhoeological activity, thought to be based on its ability to reduce blood viscosity and increase the filterability of blood cells. While several studies in animal models have suggested that pentoxifylline is able to decrease the cytokine-induced toxicity of anti-neoplasic agents, while preserving anti-tumour treatment efficacy [55], clinical studies have shown that the drug failed to improve appetite or to increase the weight of cachectic patients [56]. Rolipram is a type IV phosphodiesterase inhibitor that decreases TNF-α production by lipopolysaccharide (LPS)-stimulated human monocytes. This compound was previously used in the treatment of endogenous depression in animals and humans, and it may have therapeutic activity in disease states in which TNF-α seems to play a role in the pathogenesis, such as in endotoxic shock. Thalidomide (α-N-phthalimidoglutaramide) is a drug unfortunately associated with tragedy. Indeed, its use as a sedative in pregnant women caused over 10000 victims of severe malformations in newborn children. However, use of the drug has undergone a revival since it was demonstrated to suppress TNF-α production in monocytes in vitro and to normalise elevated TNF-α levels in vivo. Its use in cancer cachexia remains to be established but it may have a specific role in counteracting TNF-α-mediated metabolic changes [57].

The use of anti-cytokine antibodies (either monoclonal or polyclonal) and cytokine receptor antagonists or soluble receptors has led to very interesting results. Thus, in rats bearing the Yoshida AH-130 ascites hepatoma (a highly cachectic tumour), anti-TNF-α therapy resulted in a partial reversal of the abnormalities associated with both lipid and protein metabolism [58]. In humans, however, clinical trials using anti-TNF-α treatment have led to poor results in reverting the protein wasting associated with sepsis [59]. Concerning IL-6, experimental models have proved that the use of antibodies is highly effective in preventing tumour-induced wasting. Strassman et al. [14] demonstrated that the experimental drug suramine (which prevents the binding of IL-6 to its cell-surface receptor, as demonstrated by radioreceptor binding assays and affinity binding experiments) partially blocks the catabolic effects associated with the growth of colon-26 adenocarcinoma in mice. In humans, administration of an anti-IL-6 monoclonal antibody to patients with AIDS and suffering from an immunoblastic or a polymorphic large-cell cell lymphoma had a highly positive effect on fever and cachexia. Concerning other cytokines, anti-IFN-γ therapy has also been effective in reverting cachexia in mice bearing the Lewis lung carcinoma [18], but clinical data are lacking. It has to be pointed out here that the routine use of anti-cytokine antibodies is, at present, too expensive, due to the fact that this type of therapy requires a very large number of antibody molecules in order to completely block cytokine action.

The appearance of the cachectic syndrome is dependent not only on the production of the above-mentioned cytokines, known as catabolic pro-inflammatory cytokines, but also on so-called anti-inflammatory cytokines (Fig. 1), such as IL-4 and IL-10. Mori et al. [60] demonstrated that administration of IL-12 to mice bearing colon-26 carcinoma alleviated the body weight loss and other abnormalities associated with cachexia, such as adipose tissue wasting and hypoglycaemia. The anti-cachectic properties were obtained at low doses of IL-12, insufficient to inhibit tumour growth. The effects of IL-12 seem to be dependent on an important decrease of IL-6, a cytokine responsible for cachexia associated with this tumour model. A similar action was described for INF-α. Administration of this cytokine promoted a decrease in IL-6 mRNA expression in the tumour and in serum IL-6 levels, resulting in the amelioration of cachexia in a murine model of malignant mesothelioma. IL-15 has been reported to be an anabolic factor for skeletal muscle [61], and experiments carried out in our laboratory clearly demonstrate that the cytokine is able to reverse most of the abnormalities associated with cancer cachexia in a rat tumour model [62].

Additional anti-inflammatory strategies to influence cytokine levels during cachexia include the use of cyclooxygenase-2 inhibitors [63, 64]. These compounds, in addition to decreasing cytokine levels in cancer, result in an improvement in weight loss and cachexia.

Concerning therapeutic strategies based on events related to transcription factors in muscle wasting, several points can be raised. First, Kawamura et al. [65, 66] reported an oligonucleotide that competes with a NFκB-binding site reverts cachexia in a mouse experimental model, without affecting growth of the primary tumour. This treatment, however, reduces the metastatic capacity of colon-26 adenocarcinoma. In spite of this, administration of curcumine to tumour-bearing rats was unable to block muscle wasting, implying that NFκB is not involved in the cachectic response in this tumour model [48].

As noted above, AP-1 is clearly involved in muscle wasting during sepsis [45] and in cancer (unpublished data). Interestingly, administration of an inhibitor of NFκB and AP-1 resulted in a partial blockade of muscle wasting in rats bearing the AH-130 Yoshida ascites hepatoma, a highly cachectic rat tumour (unpublished data).

Conclusions

Since metabolic alterations often appear soon after the onset of tumour growth, the scope of appropriate treatment, although not aimed at achieving immediate eradication of tumour mass, could influence the course of the patient's clinical state or, at least, prevent the steady erosion of dignity that the patient may feel in association with the syndrome. This would no doubt contribute to improving the patient's quality of life and, possibly, prolong survival. Although exploration of the role that cytokines play in the host response to invasive stimuli is an endeavour that has been underway for many years, considerable controversy still exists over the mechanisms of lean-tissue and body-fat dissolution that occur in the patient with either cancer or inflammation, and whether humoural factors regulate this process. A better understanding of the role of cytokines, both host and tumour-derived [32], in the molecular mechanisms of protein wasting in skeletal muscle, is essential for the design of effective therapeutic strategies. In any case, understanding the humoural response to cancer and modifying cytokine actions pharmacologically may prove very suitable, and no doubt future research will concentrate on this interesting field. Finally, understanding the intracellular signalling mechanisms, particularly those involving transcriptional factors, may also be very important for achieving effective therapeutic approaches.

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