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Review

Cancer Research

Concomitant Tumor Resistance: The Role of Tyrosine Isomers in the Mechanisms of Metastases Control

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Abstract

Concomitant tumor resistance (CR) is a phenomenon in which a tumor-bearing host is resistant to the growth of secondary tumor implants and metastasis. Although previous studies indicated that T-cell–dependent processes mediate CR in hosts bearing immunogenic small tumors, manifestations of CR induced by immunogenic and nonimmunogenic large tumors have been associated with an elusive serum factor. In a recently published study, we identified this factor as *meta*-tyrosine and *ortho*-tyrosine, 2 isomers of tyrosine that would not be present in normal proteins. In 3 different murine models of cancer that generate CR, both *meta*- and *ortho*-tyrosine inhibited tumor growth. Additionally, we showed that both isoforms of tyrosine blocked metastasis in a fourth model that does not generate CR but is sensitive to CR induced by other tumors. Mechanistic studies showed that the antitumor effects of the tyrosine isomers were mediated in part by early inhibition of the MAP/ERK pathway and inactivation of STAT3, potentially driving tumor cells into a state of dormancy in G_0-phase. Other mechanisms, putatively involving the activation of an intra–S-phase checkpoint, would also inhibit tumor proliferation by accumulating cells in S-phase. By revealing a molecular basis for the classical phenomenon of CR, our findings may stimulate new generalized approaches to limit the development of metastases that arise after resection of primary tumors or after other stressors that may promote the escape of metastases from dormancy, an issue that is of pivotal importance to oncologists and their patients. *Cancer Res; 72(5); 1043–50. ©2012 AACR.*

Introduction

Concomitant tumor resistance (CR) is a phenomenon in which a tumor-bearing host inhibits or retards the growth of secondary tumor implants. It was first described by Ehrlich in 1906 (1), but apart from a few isolated studies (2, 3), this phenomenon remained virtually forgotten for ~60 years (4–6). After a renewal of interest in this concept, some groups studied it primarily by using tumor models in mice, rats, and hamsters (4, 7, 8). However, CR has not received as much attention as other areas of cancer research, despite the fact that it has been detected in association with human cancer and despite its relevance to the mechanisms of metastases control.

Resistance of cancer patients to reinoculation of autologous tumor cells was originally described by Southam (5) and Brunswig and colleagues (6). In their experiments, they obtained tumor cells from patients with cancers of the ovary or uterus, and they inoculated autologous tumor cells at determined sites on the anterior region of the thigh. The results showed that the antitumor resistance to autotransplantation was more profound in patients with localized cancer than in those with regional or distant metastases.

Concerning the relevance of CR for mechanisms of metastasis control, it has been observed that the removal of human and murine tumors may be followed by an abrupt increase in metastatic growth (9–16). This suggests that in certain circumstances, the primary tumor exerts a controlling action on its metastases, which can be considered as secondary tumor implants that developed spontaneously during the primary tumor growth.

In the experimental setting, accelerated growth of spontaneous metastases after excision of the primary tumor was observed almost a century ago by Tyzzer (17). He observed that although the surgical removal of a primary murine tumor prolonged the survival of mice, the size of the developed metastatic nodules was larger than in mice bearing the primary tumor. Similar results were obtained by Tadenuma and Okonogi (18) in 1924. In the last 50 years, different groups have confirmed and extended those pioneer experiments by studying the growth of spontaneous and experimentally induced metastases in tumor-bearing hosts (11–13, 19–22). A rather general pattern derived from these experiments was reviewed previously (11, 20) and can be summarized as follows: The outcome of the removal of a subcutaneous metastatic tumor depended on the size of the local tumor removed. When small tumors were surgically excised, the lungs were left with very few metastatic cells compared with the number in the lungs of tumor-bearing mice in which the primary tumor continued to shed numerous cells into the circulation. As a consequence, the

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total mass of proliferating metastatic cells in tumor-bearing mice exceeded the growth of the fewer cells existing in the lungs of the tumor-excised mice. At this stage, tumor excision significantly prolonged the survival of the mice. When medium-sized tumors were removed, equilibrium could be reached between the effect of suppression exerted by the primary tumor and the shedding of potentially metastatic cells. Consequently, the total mass of proliferating metastatic cells was similar in both tumor-bearing and tumor-excised mice, because although the tumor-excised mice displayed fewer lung metastatic foci, each focus was of a larger size. At this stage, tumor removal still (albeit modestly) prolonged the survival of the operated mice, presumably because even though both metastatic lung masses were similar, the presence of the primary growing tumor was deleterious for the health of the host. Finally, when large tumors were removed, a higher level of proliferating metastatic cells and larger metastatic nodules compared with those present in tumor-bearing mice were observed. At this stage, tumor excision resulted in a significantly reduced survival rate in the group of operated mice.

In clinical settings, an accelerated growth of metastases following tumor resection has been suspected for decades (23). However, studies comparing metastatic growth in patients with nonexcised tumors (expectant management) with that in patients who have undergone tumor resection (surgical management) are required to definitively show such an effect. Because surgery is one of the primary treatment modalities for solid cancers, such studies are not performed frequently; however, a few can be found in the literature. For example, Iversen and colleagues (24) found no benefit with radical prostatectomy over expectant management in a 23-year follow-up study of 111 patients with adenocarcinoma of the prostate. Similarly, Demicheli and colleagues (25, 26) examined death-specific hazard rates in a group of patients with breast cancer who had undergone mastectomy alone in comparison with nonoperated patients obtained from an accepted historical database. The group of nonoperated patients (expectant management) exhibited a single peak between the fourth and fifth years in the hazard rate for death. In contrast, a 2-peak hazard was detected in the group of operated patients. The first peak occurred between the third and fourth years after surgery, followed by a second peak in the eighth year. Similar patterns of tumor recurrence after mastectomy were observed by other investigators (27), suggesting that the natural history of breast cancer may be adversely affected in some way by removal of the primary tumor.

In many other types of cancer, because of a lack of nonoperated control patients, investigators have been unable to definitively show an enhancement of regional and distant residual tumor growth (i.e., metastases) after primary tumor removal. However, a significant body of evidence that has accumulated over the last 40 years points in that direction. For example, Sugarbaker and colleagues (9) reported a clinical case of a 26-year-old male with a melanoma in the scalp. The disease was clinically localized, and evaluation revealed no disseminated metastases. A wide excision and graft were performed, and numerous subcutaneous nodules as well as visceral metastases appeared 6 weeks postoperatively. Lange and colleagues (10) reported a study of 8 patients who underwent cytoreductive surgery for testicular cancer. In each case, tumor cytoreductive surgery led to a much faster growth of regional and distant residual disease than would be expected by assuming an uninterrupted, natural growth of residual tumors that were not apparent at the time of surgery. Similar findings in patients with epithelial ovarian cancer (28) led some investigators to urge caution with respect to cytoreductive surgery (28, 29).

The above clinical studies, together with similar investigations carried out with patients affected by similar or other malignancies, strongly suggest that sudden acceleration of metastases may be an undesired outcome of surgical removal of many common human malignancies, including primary melanomas and breast, testicular, ovarian, lung, colorectal, and bladder cancers (9, 10, 16, 23–33).

On the other hand, the phenomenon of concomitant enhancement, in which the presence of a primary tumor stimulates the growth of metastases, has also been observed (34, 35). However, in our experience (36), the magnitude of this stimulatory effect, when present, proved to be rather modest compared with the magnitude of the inhibitory effect produced by CR.

Mechanisms Proposed to Explain the Phenomenon of CR

As noted above, most experimental and clinical reports in the literature provide strong evidence that the process of tumor removal adversely alters the fate of minimal residual disease locally (local recurrence) and systemically (metastasis). In fact, local recurrence and especially metastatic growth are far more serious problems than the original tumor because, in most cases, they ultimately prove to be fatal for the patient. Given that the growth of tumor cells reinoculated into animals bearing a primary tumor mimics the situation observed during metastases formation, it appears that an understanding of the mechanisms underlying the phenomenon of CR could provide insight into the mechanisms that inhibit the growth of metastatic cells in the presence of a primary tumor. This in turn would aid in the design of new strategies to limit the development of metastases that arise after resection of primary tumors.

Different hypotheses have been proposed to explain the phenomenon of CR. According to the immunological hypothesis, the growth of a tumor generates a specific antitumor immune response that may not be strong enough to inhibit the primary tumor growth but will still be able to prevent the development of a relatively small secondary tumor inoculum. This explanation is not very different from that of conventional immunologic rejection of allogeneic tumors in naive mice or immunogenic syngeneic tumors in previously immunized animals. The immunological hypothesis was originally proposed in 1908 by Bashford and colleagues (2), who also coined the term "concomitant immunity," by which this phenomenon was known in the past. This interpretation is supported by solid evidence mainly based on experiments with strongly

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immunogenic murine tumors induced by chemical agents or viruses (8, 37). However, it does not provide a satisfactory explanation for the fact that CR has also been observed in association with spontaneous murine tumors of nondetectable immunogenicity (38, 39).

Nonimmunological explanations rely mainly on 2 hypotheses. Ehrlich (1) and Tyzzer (17) believed that nutrients essential for tumor growth are consumed by the primary tumor, making it difficult or impossible for a second implant to develop (the atrepsis theory).

Other investigators (20, 39–42) have postulated that tumor cells of the primary tumor produce (or induce the production of) antiproliferative nonspecific substances or antiangiogenic molecules that suppress or limit, directly or indirectly, the replication of tumor cells of the second inoculum.

These nonimmunological hypotheses can offer a putative explanation for the phenomenon of CR induced by nonimmunogenic tumors but not for the specific inhibition of secondary tumor implants observed during the growth of immunogenic tumors.

For the last 25 years, our laboratory has studied the phenomenon of CR associated with the growth of 17 murine tumors with widely different degrees of immunogenicity, in an attempt to integrate the different hypotheses into a coherent picture.

Our results (8, 39, 43–45) describing 2 temporally separate peaks of CR during primary tumor growth may explain many apparently contradictory results reported by different authors (3, 8, 20, 37). In our opinion, these differences are related to the different stages of tumor growth at which these authors looked for CR, as well as to the different characteristics of both peaks. In effect, the first peak was observed when the primary tumor was small (<500 mm^3). It was tumor-specific and thymus-dependent, as it was exhibited in euthymic but not in nude mice, and its intensity was proportional to tumor immunogenicity. A typical immunological rejection, associated with extensive necrosis and a profuse infiltration with polymorphonuclear granulocytes and mononuclear cells, was observed histologically at the site of the second tumor implant undergoing CR. Furthermore, the kinetics of appearance and disappearance of the first peak of CR paralleled the kinetics of appearance and disappearance of cytotoxic antibodies and cell-mediated cytotoxicity against the tumor.

On the other hand, the second peak of CR was induced by both immunogenic and nonimmunogenic large tumors ($\geq$2,000 mm^3). It was not tumor-specific or thymus-dependent, as it was exhibited in both euthymic and nude mice, and it did not correlate with tumor immunogenicity. Inhibition of the secondary tumor in the presence of a large primary tumor was not associated with a massive or focal necrosis, or with any host cell infiltration, but it was associated with the presence of noninfiltrating tumor cells (i.e., dormant tumor) located at the inoculation site between the skin and the muscular layer.

Some years ago, an intermediate peak of CR was reported to be associated with a particular type of mid-sized tumors (1,000–1,500 mm^3) that restrain secondary tumors indirectly by limiting tumor neovascularization (41).

Although the mechanisms associated with the first and intermediate peaks of CR have been elucidated as T-cell–dependent and angiostatin-dependent, respectively, the molecular basis of the most universal manifestation of CR (i.e., the second peak) has remained an enigma for many years.

In previous studies, we showed that the second peak of CR correlated with the activity of a serum factor(s), different from antibodies or complement, that inhibited the *in vitro* and *in vivo* proliferation of tumor cells. When this serum inhibitory activity was absent (the only 2 cases were mice bearing highly metastatic C7HI and MM3 mammary adenocarcinomas), the second peak did not appear. These results suggested a direct correlation among the second peak of CR, the capacity to restrain metastatic growth, and the titer of serum growth-inhibitory activity. Furthermore, lung metastases produced by C7HI and MM3 tumors were significantly inhibited by both the concomitant presence of unrelated tumors that induced CR and the daily administration of serum from mice bearing these unrelated tumors, which displayed a high titer of growth-inhibitory activity (12, 13).

We partially characterized this inhibitory activity in our laboratory and obtained a heat-, acid-, and alkali-resistant factor of low molecular weight that apparently was unrelated to other well-characterized, growth-inhibitory molecules (e.g., interferons, TNF-α, TGF-β, angiostatin, and endostatin), taking into account the larger molecular weight of the latter and other physical and biological properties (13, 39, 44).

However, despite these efforts, the origin and chemical nature of that factor remained elusive for years. In addition, the question of how such a factor could inhibit the proliferation of a secondary tumor but not that of a large primary one composed of the same type of cells remained unresolved.

In a recent study of mice bearing a nonimmunogenic lymphoma (called LB) that produced the strongest second peak of CR of all of our tumor models, we reported the origin, isolation, and identification of the serum factor(s) associated with the phenomenon of CR (46). We also reported its biological antitumor activity and the putative mechanisms of tumor inhibition.

Tyrosine isomers mediate the most universal manifestation of CR

The task of characterizing the serum factor(s) associated with the second peak of CR was long and difficult due to the very low concentration of the active molecule(s) and the overwhelming amount of tyrosine (Tyr) present in the purified antitumor serum fraction, which masked the existence of other molecules and considerably retarded the process of characterization. The elucidation of this puzzle was achieved when, after several steps of purification (see Fig. 1), we finally detected minimal amounts of *meta*-tyrosine (*m*-Tyr) and *ortho*-tyrosine (*o*-Tyr), 2 isomers of Tyr that are thought to be absent from normal proteins, together with Tyr using high-resolution ion-electrospray mass spectrometry and tandem mass spectrometry. We identified *m*- and *o*-Tyr as being responsible for 90% and 10%, respectively, of the total antitumor activity, as shown by *in vitro* and *in vivo* experiments on the growth of LB and other 2 murine tumors (MC-C fibrosarcoma

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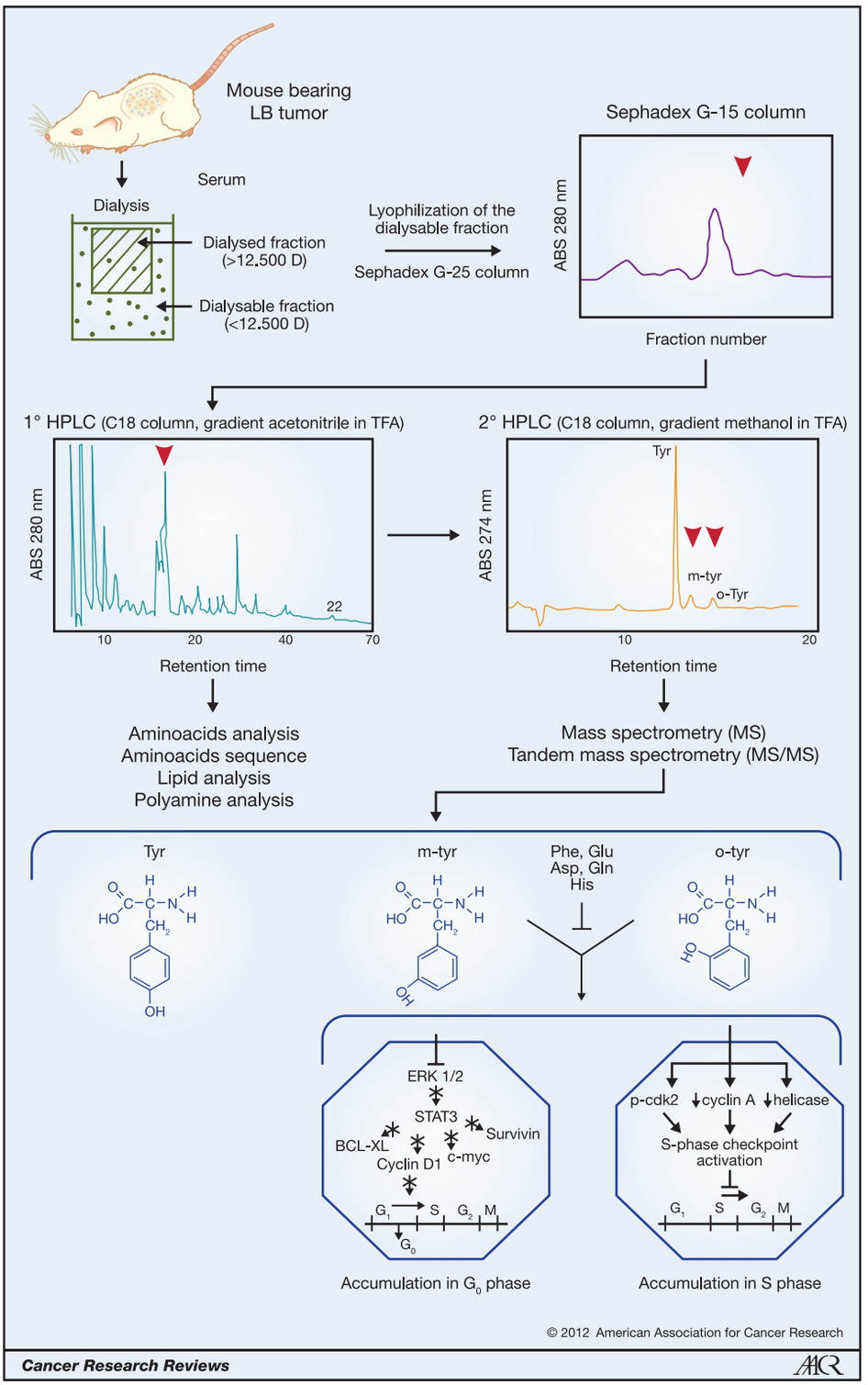

Figure 1. General schedule of purification of the antitumor serum factor(s) associated with the phenomenon of CR and putative/speculative mechanisms of tumor inhibition. The arrowhead (▼) indicates the fractions with antitumor activity through the different steps of purification. The antitumor activity produced by *m*-Tyr was about 10 times more robust than that produced by *o*-Tyr. Conventional Tyr did not produce any antitumor effect. The amino acids Phe, Glu, Asp, and Gln counteracted the antitumor effects mediated by both *m*- and *o*-Tyr, whereas His only counteracted the effect mediated by *m*-Tyr. The arrow (↓) indicates activation, and the symbol (⊥) indicates inhibition or inactivation. BCL-XL, B-cell lymphoma XL; cdk2, cyclin-dependent kinase 2; ERK 1/2, extracellular signal-regulated kinases 1 and 2; *p*-cdk2, phosphorylated or inactive cdk2; STAT3, signal transducer and activation of transcription 3; TFA, trifluoroacetic acid.

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and CEI epidermoid carcinoma) that induce CR, and on the growth of established spontaneous metastases generated by a highly metastatic mammary adenocarcinoma (C7HI) that does not induce CR but is sensitive to the CR induced by other tumors. The tumor inhibitory effects produced *in vitro* by *m*- and *o*-Tyr were rapidly detectable after 8 to 18 hours in culture even at low (micromolar) concentrations. Those produced *in vivo* were observed, without exhibiting any toxic side effects, not only on tumor implants but also on growing vascular (subcutaneous) and avascular (ascitic) tumors, suggesting that they may have therapeutic potential based on a direct effect on tumor cells rather than an indirect effect on tumor vascularization.

The inhibition exerted by *m*- and *o*-Tyr on tumor growth mimics the inhibition produced by CR. In both cases, tumor inhibition was associated with the presence of a high proportion of cells in G_0, a decrease in G_2–M phases, and an increase of the S-phase, which was considered to be the consequence of an S-phase arrest. In addition, both a secondary tumor inhibited by CR and a tumor inhibited by exogenous injection of *m*-Tyr were able to rapidly resume their growth when transplanted in a normal mouse or when treatment with *m*-Tyr was interrupted, respectively.

The inhibitory effect produced *in vivo* and *in vitro* by *m*-and *o*-Tyr on tumor cell proliferation was counteracted by phenylalanine (Phe) and, to a lesser degree, glutamic acid (Glu), aspartic acid (Asp), glutamine (Gln), and histidine (His), but not by Tyr or the remaining protein amino acids.

Central Paradox of CR

The central paradox of CR, i.e., the inhibition of secondary tumor implants together with the progressive growth of the primary tumor, has remained unresolved for more than a century. To account for this problem, we showed that as a primary tumor grows, relatively large amounts of most amino acids, including those that counteract the inhibitory effects of *m*- and *o*-Tyr (i.e., Phe, Glu, Asp, Gln, and His), accumulate in the tumor microenvironment, whereas at distant sites, such as sites of putative secondary tumor implants, the content of amino acids is significantly lower. Furthermore, a cocktail of amino acids similar to that detected close to the primary site proved to be more counteracting of the antitumor effects produced by *m*-and *o*-Tyr than a cocktail similar to that detected at a secondary site. On this basis, we suggested that a secondary tumor can be inhibited by circulating *m*- and *o*-Tyr, while at the same time, the primary tumor is protected from their inhibitory effects, at least in part, by these counteracting amino acids and thus can continue to grow. This suggestion seems to reconcile the 2 major nonimmunological interpretations of CR that have been advanced to date, i.e., the antiproliferative-factors hypothesis and the atrepsis theory (1, 17, 20, 39–41). In effect, the postulation that serum *m*- and *o*-Tyr are responsible for the inhibitory effect generated by a primary tumor on the growth of secondary tumor implants is similar to the hypothesis of antiproliferative factors. However, the mere presence of inhibitory factors such as *m*-and *o*-Tyr is not enough to explain why the primary tumor can grow while the secondary one cannot. On the other hand, the different concentration of amino acids at the site of the primary tumor compared with other parts of the organism would appear to support the atrepsis theory because, according to this theory, the primary tumor accumulates elements that allow it to grow and whose lack at distant sites from the primary tumor will prevent a second tumor from growing. However, whereas according to the atrepsis theory, these elements are nutrients that would directly stimulate growth of the primary tumor, in our postulation, these elements would allow the primary tumor to grow by counteracting the effect of circulating inhibitory factors. Some years ago, Prehn (47) anticipated this interpretation and suggested that CR could best be explained by the competitive interaction of 2 opposing (and up to that time uncharacterized) influences: a local, slowly diffusible, tumor-facilitating environment that would be counteracted by circulating inhibitors.

The intriguing observation that regeneration of normal tissues is usually not affected in tumor-bearing mice that exhibit the second peak of CR (43, 46) might also be explained by assuming that these regenerating tissues, but not secondary tumor implants, display a content of amino acids high enough to counteract the inhibitory effects produced by *m*- and *o*-Tyr.

Origin of Tyr Isomers and Putative Mechanisms of Tumor Inhibition

To date, *m*- and *o*-Tyr have been studied almost exclusively as markers for oxidative damage associated with abnormal proteins as detected in, for example, the blood of animals subjected to cardiac ischemia-reperfusion injury, mitochondria of exercised animals, atherosclerotic tissue of diabetic primates, and aging lens of humans (48).

Most investigators have assumed that *m*- and *o*-Tyr are generated posttranslationally when the L-Phe present in proteins is exposed to hydroxyl radicals during oxidative damage. However, it was recently suggested that oxidized amino acids, such as *m*- and *o*-Tyr, may also be generated from free amino acids that subsequently could be incorporated into proteins during synthesis (48, 49). We previously observed that the serum antitumor activity attributed to *m*- and *o*-Tyr was strongly inhibited by agents that reduced the number of myeloid-derived suppressor cells (MDSC) and oxidative damage, and that in tumor-bearing mice (including the LB tumor model used in this work) and some cancer patients, MDSCs that produced large amounts of reactive oxygen species accumulated progressively in circulation (46, 50–53). On the basis of these results, we suggested that free *m*- and *o*-Tyr present in the serum from tumor-bearing mice will be produced, at least in part, when circulating molecules of Phe are oxidized by hydroxyl radicals released by MDSC.

Very few investigators have reported antiproliferative effects mediated by *m*- and *o*-Tyr. Gurer-Orhan and colleagues (48), while studying alternative mechanisms for oxidative stress and tissue injury during aging and disease, showed that free *m*-Tyr and *o*-Tyr were toxic to Chinese hamster ovary cells when the cells were incubated *in vitro* with *m*- or *o*-Tyr for 7 to 10 days. In the same way, Bertin and colleagues (49), while studying the

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development of more environmentally friendly weed-management systems, showed that the unusual ability of many fine fescue grasses to outcompete or displace other neighboring plants was based on the phytotoxic properties of their root exudates, and that >80% of the active fraction was *m*-Tyr. Both groups hypothesized that a possible cytotoxicity mechanism could involve mischarging of tRNA and consequent misincorporation of these unnatural isomers of Tyr into cellular proteins based on their structural similarities with Phe or Tyr. In turn, this misincorporation could cause a structural disruption in proteins or interfere with the functions of key enzymes, such as DNA polymerase, that might lead to errors in DNA replication and long-term consequences such as impaired cellular viability.

The mechanism of misincorporation into cellular proteins, claimed to be associated with long-lasting cytotoxicity effects on mammal and plant normal cells, could also be invoked to explain the short-lasting antiproliferative effects of *m*- and *o*-Tyr on tumor cells described in our previous work (46). Although this alternative is possible, some of their antitumor effects might start before such a misincorporation in proteins has a chance to occur. This is suggested by the rapid reversion of those effects, as well as by the counteracting effects of amino acids (other than Phe) that lack any obvious structural similarity to *m*- and *o*-Tyr and consequently have fewer possibilities to compete for the same tRNA. Furthermore, a molecular analysis showed that the antitumor effects mediated by *m*- and *o*-Tyr were mediated, at least in part, by a very early inhibition of the MAP/ERK signaling pathway that would drive tumor cells into a state of dormancy through a rapid decay of *p*-STAT3 (46). The intimate mechanisms by which a partial inactivation of *p*-STAT3 could drive tumor cells into a state of dormancy remain speculative. We suggest that downregulation of the expression of several genes engaged with cell proliferation and survival that are targets of STAT3, such as BCL-XL (B-cell lymphoma XL), cyclin D1, survivin, and myc, among others, might induce tumor cells to enter into G_0-phase. The arrest of many tumor cells in G_0, as revealed by the low expression of Ki-67, a protein that is expressed in G_1–M but not in G_0 (46), is a fact and could be a consequence of the partial inactivation of STAT3. However, another feature has also been systematically associated with the inhibitory effects mediated by CR *in vivo* and by *m*- and *o*-Tyr in *in vivo* and *in vitro* settings: the accumulation of a fraction of tumor cells in S-phase (44, 46). This S-phase arrest may be generated by a mechanism different from that associated with STAT3 inactivation. Several factors and conditions, such as resveratrol (54), hyperoxia (55), hydroxyurea (56), ultraviolet radiation (57), G-rich oligonucleotides (58), and zidovudine (59), induce the inhibition of cell proliferation associated with an S-phase arrest, presumably by the activation of an intra–S-phase checkpoint. Different mechanisms for activating this checkpoint have been proposed, including accumulation of cdk2 (cyclin-dependent kinase 1) in its inactive phosphorylated form (54, 55), downregulation of cdk2 (56), activation of ATM/ATR (ataxia telangiectasia mutated/ataxia telangiectasia Rad 3-related) kinase in response to DNA damage (57), modulation or inhibition of a replicative helicase activity (58), and downregulation of cyclin A2 (59). Some of these pathways may also be involved in the S-phase arrest mediated by *m*- and *o*-Tyr (Fig. 1).

It is clear that we need a more profound understanding of the molecular mechanisms of tumor inhibition by *m*- and *o*-Tyr. From an evolutionary point of view, it is interesting that when *m*-Tyr is present in root exudates from many fescue grasses, it can inhibit the growth of competing neighboring plants, and when it is present in tumor-bearing mice, it can inhibit the growth of secondary tumor implants.

Two intriguing questions

The above-mentioned mechanistic considerations raise 2 intriguing questions. The first one can be stated as follows: If MDSCs produce *m*- and *o*-Tyr that inhibit, at least in part, the proliferation of secondary tumors by STAT3 inactivation, why do *m*- and *o*-Tyr not inhibit the expansion of MDSCs that have been shown to be STAT3-activation–dependent (60)? Although we do not have a definitive answer for that question, we can suggest an explanation based on our interpretation of the central paradox of CR. We may assume that a high content of amino acids that counteract the antitumor effects mediated by circulating *m*- and *o*-Tyr, similar to that found at the site of a primary tumor and presumably also to that found in normal regenerating tissues from tumor-bearing mice (46), could be present in the bone marrow, where proliferation of myeloid progenitors and generation of MDSCs occur. If this were the case, we could understand why the abnormal proliferation of myeloid progenitors that generate the expansion of MDSCs would be protected from the inhibitory effects mediated by *m*- and *o*-Tyr, while at the same time, a secondary tumor (where the content of these counteracting amino acids proved to be low) would be inhibited.

The second intriguing question concerns the relationship between the MAP/ERK pathway and the transcriptional activity of STAT3, which has been suggested to be involved, at least in part, in the inhibitory effect of *m*-Tyr on tumor cell proliferation. Activation of the MAP/ERK pathway produces STAT3 phosphorylation in serine 727; however, this phosphorylation does not by itself induce STAT3 transcriptional activity. Instead, STAT3 transcriptional activity is associated with Tyr phosphorylation (Y-705), which is induced by activation of JAKs, EGFR, and other Tyr kinase-related pathways. These apparently contradictory statements can be reconciled by previous experiments showing that cooperation of both Tyr and serine phosphorylation is necessary for full activation of STAT3 (61–63). Consequently, diminishing serine phosphorylation in STAT3 by inactivation of the MAP/ERK pathway produced by *m*-Tyr could actually induce a decreasing STAT3 transcriptional activity and then an inhibition of tumor cell proliferation.

Conclusions and Perspectives

Surgical extirpation is the mainstay treatment of solid tumors and may be curative when metastatic cells have not already disseminated from the primary tumor. However, although it is recommended in most clinical cases, tumor removal entails an undesired side effect: the acceleration of

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regional and distant (metastases) residual neoplastic disease. This effect may account for the disappointingly modest survival benefits observed when surgery is used as a single strategy of treatment. Investigators have proposed some therapeutic options to limit metastatic growth after tumor removal, including the use of perioperative (instead of postoperative) chemotherapy, antioxidant agents, immunotherapy, and biomodulation (29), but to date, the results have not been as promissory as expected.

An understanding of the phenomenon of CR could help us overcome this problem. However, CR has largely been neglected by researchers and clinicians, probably because the idea that a primary tumor can exert inhibitory influences on distant metastases meant that a tumor had to be considered an integrated, organ-like entity rather than a collection of independent atypical cells. In fact, hepatectomy stimulates mitosis in previously resting ectopic implants of hepatocytes in the same way that excision of a primary tumor induces mitosis in previously arrested secondary tumor implants (39, 64, 65). This and many other examples of CR-like phenomena associated with normal tissues and organs suggest that a tumor may mimic some aspects of organ homeostasis (23, 47, 66, 67). Along with this new conceptual model of cancer, we recently showed that *m*- and *o*-Tyr are responsible for the most universal manifestation of CR (46). For therapeutic purposes, both of these Tyr isomers, which have been preserved throughout evolution as antiproliferative factors in 2 different biological kingdoms, have many attractive features. For example, they exert their effects even at very low concentrations, are naturally produced in the proper tumor-bearing organism, are easily available, and do not exhibit any detectable toxic side effects even when the highest therapeutic dose is used.

By revealing a molecular basis for the antitumor effects mediated by *m*- and *o*-Tyr, our findings may help unveil some of the control mechanisms of malignant and normal cell proliferation. They may also aid in the development of new and less harmful means of managing malignant diseases, especially by controlling the growth of metastases after the removal of a primary tumor, or after other surgical injuries or stressors that may promote the escape of metastases from dormancy (23, 68, 69). We believe this is an issue of pivotal importance to oncologists and their patients.

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