The Pathway to Biomarker Discovery: Carbonic Anhydrase IX and the Prediction of Immune Responsiveness

Commentary on Atkins M et al., p. 3714

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Relevance of the Question

The level of carbonic anhydrase IX-G250MN (CAIX) expression in renal cell carcinoma (RCC) tissue samples may serve as the first useful biomarker predictive of a response to interleukin 2 (IL-2) therapy (1). IL-2 is the only biological agent approved by the U.S. Food and Drug administration for the treatment of stage IV RCC because it can induce in a small percentage of patients’ durable complete responses (2). The use of IL-2 is limited by severe toxicity and cost; therefore, the therapeutic value of the drug would be enhanced by the ability to improve the risk/benefit ratio of its administration by predicting which patients may or may not be responsive. Recent efforts have been focused in identifying biomarkers predictive of response to therapy that could be used for patients’ selection. The level of CAIX expression in RCC tissue samples may serve as the first useful predictive marker (1) because the association of most other factors with clinical response is not universally accepted (2) and for several of them it is treatment related (i.e., toxicities) and therefore cannot be used for patient selection (2, 3). In particular, when the CAIX-based model was refined by stratifying patients according to other favorable or unfavorable pathologic features, the prediction was almost faultless (see Table 4 in Atkins et al.; ref. 1). Whereas the results of this study should encourage the choice of systemic IL-2 therapy for patients with CAIX expressing tumors, we agree with Atkins et al. (1) that the overlap in response rates observed among patients with different levels of CAIX expression should not justify its use as an absolute criterion for patients’ exclusion from treatment pending further independent and prospective validation. In addition, relationships have been described between toxicity and effectiveness of systemic IL-2 administration suggesting a common effector pathway (4, 5). Therefore, it would be interesting to know in the future whether CAIX is associated with some of the IL-2 related toxicities or it is an independent predictor of responsiveness. Obviously, the ability to segregate the mechanism(s) responsible for these two biological effects of IL-2 may suggest ways of administration as single agent or in combination therapy to increase the rate of responses whereas decreasing toxicity.

The Biology Behind

The mechanism(s) at basis of clinical regressions in response to systemic IL-2 administration remain elusive. In fact, we observed that the in vivo activity of this biological response modifier seems to differ from its predicted effect on T-cell stimulation. IL-2, instead, mediates an activation of innate effector mechanism through the downstream release of an array of cytokines by circulating and/or intratumoral immune cells (7, 9). Transcriptional analysis of melanoma metastases in a relatively small cohort of patients undergoing combined antigen-specific immunization and systemic IL-2 administration suggested that immune responsive lesions are immunologically different even before treatment from immune-resistant lesions because they express immunologic signatures consistent with chronic inflammation (10). IL-2 may act through the downstream release of soluble factors including cytokines and chemokines (9, 11) that may turn a chronic inflammatory process into an acute one through activation of innate immune effector functions (7). In particular, this acute inflammation may act as a costimulatory signal for tumor antigen-specific T cells that have reached the tumor site and are exposed to antigen stimulation by interactions with tumor cells or immature mononuclear phagocytes (12, 13). However, confirmed biomarkers of immune responsiveness are not available in the context of either melanoma or RCC.

How Does CAIX Fit the Picture?

In this issue of Clinical Cancer Research, Atkins et al. (1) expand on previous observations (2, 14) suggesting that CAIX...
is a favorable prognostic marker for RCC and its constitutive expression in primary or metastatic lesions may be a predictor of immune responsiveness to IL-2 therapy (14). We recently noted by gene profiling of tumors of various histology that the expression of CAIX is mostly confined to RCC (15). However, CAIX was sporadically expressed by other epithelial tumors but never by normal kidneys suggesting that its transcriptional activation, contrary to that of CAII, is not patient dependent but rather associated with the oncogenic process. This is in line with our observation in melanoma that immune responsiveness is predetermined before treatment and seems related primarily to the biology of the tumors rather than the genetic background of the patient (10). Interestingly, in the same study, none of seven metastases of melanoma, another cancer highly responsive to systemic IL-2 administration, were found to express CAIX. This finding was confirmed more recently in a larger data set where 59 melanoma lesions were compared with >100 tumors of other histology (16). This surprising finding suggests that the upstream pathways leading to immune response in melanoma may be different from that of CAII, is not patient dependent but rather associated with the oncogenic process. This is in line with our observation in melanoma that immune responsiveness is predetermined before treatment and seems related primarily to the biology of the tumors rather than the genetic background of the patient (10). Interestingly, in the same study, none of seven metastases of melanoma, another cancer highly responsive to systemic IL-2 administration, were found to express CAIX. This finding was confirmed more recently in a larger data set where 59 melanoma lesions were compared with >100 tumors of other histology (16). This surprising finding suggests that the upstream pathways leading to immune response in melanoma may be different from that of RCC if CAIX bears more than a coincidental association with immune responsiveness. It is also possible that CAIX acts upstream of a possible common denominator pathway for immune rejection of melanoma and RCC tumors. We hypothesize that the final pathway leading to tumor rejection may be similar between the two cancers and in fact may bear similarities to the biological pathway leading to rejection of basal cell carcinoma by toll receptor agonists (17) which seem synergistic with IL-2 (18). In addition, similarities seem to exist between IL-2–induced tumor regression (7) and the mechanism(s) inducing allograft rejection (8) or maintenance of systemic lupus erythematosus (19). All these biological phenomena seem to depend on the convergence in the target organ of a final pathway that seems characterized by the activation of innate immune effectors through induction of type one IFNs (2, 7, 8, 17, 19).

CAIX is an isoenzyme of the large family of carbonic anhydrases that may have redundant function. For instance, we noted that metastatic melanomas express specifically carbonic anhydrase III (muscle specific) and XIV which may have redundant function and compensate for the lack of CAIX (15, 16).

Atkins et al. (1) suggested that CAIX may maintain a balanced pH in the tumor microenvironment that may be in turn favorable for the maintenance of immune effector mechanisms. Indeed, CAIX is a hypoxia-inducible gene that participates in the VHL/hypoxia pathway (20, 21). Interestingly, unsupervised analysis of genes found up-regulated in RCC suggested that CAIX expression is tightly coordinated with that of N-myc and signal transducers and activators of transcription (STAT) interactor (NMI; Fig. 1). This gene interacts with N-myc, C-myc, and other zipper transcription factors (22). Interestingly, NMI interacts with all STATs except STAT2 and augments STAT-1–mediated transcription in response to IL-2, IFN-α, IFN-β, and IFN-γ signaling.1 In particular, the ability of NMI to interact with N-myc may be of particular significance because we have previously noted that N-myc was up-regulated in a melanoma metastasis responding to IL-2 therapy (7). In addition, CAIX expression was tightly associated with other IFN-responsive genes including IFRG28, IFI16, and C-myc and several transcription factors. This information suggests that CAIX may either play a metabolic role broader than simply maintaining extracellular ion homeostasis and may facilitate some critical functions associated with acute inflammatory processes or its expression may depend on an active immune environment as a downstream result of hypoxia-inducible factor-1α activation (23). In this regard, it is interesting to note

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1 Stroncek et al., submitted for publication.
the parallel expression of CCAAT/enhancer binding protein (C/EBP) in the CAIX cluster, because this protein is dependent on STAT-3 signaling (24) induced by oxidative stress in parallel to the expression of hypoxia-inducible factor-1α (25) responsible for the expression of CAIX (23). Therefore, it is possible that in VHL-defective tumors, even in normoxic conditions, a pseudohypoxic state is maintained which leads to the expression of transcription factors with downstream constitutive expression of CAIX, C/EBP, NML, c-myc, and other genes identified in this signature. This is a likely possibility and we do agree with the authors’ statement “CAIX may serve as a surrogate marker for some other critical hypoxia-inducible factor–mediated protein (perhaps NML) that is more directly associated with immune responsiveness (2).” The reason why this phenomenon would lead to enhanced responsiveness remains, however, elusive. A preliminary quest for functional associations among genes in the CAIX cluster based on the database for annotation visualization and integrated discovery (DAVID at http://david.niaid.nih.gov/david/upload.asp) could not identify known direct interactions between CAIX and other genes included in the signature. Similarly, CellSpace-based mining did not yield strong evidence of known associations (http://cellspace.cellomics.com/CellSpace/csmanger.asp). Therefore, extensive biological characterization of these genes function will need to be entertained in the future to better understand its relationship with immune responsiveness.

It is also possible that CAIX may be target of cellular or humoral immune responses because it is a transmembrane protein sensitive to antibody-dependent cytotoxicity (26). This may be a significant mechanism because CAIX expression is variably modulated by cytokines released in vivo in response to systemic IL-2 administration (9) such as IL-2 itself and IFNs which enhance and IL-1β and IL-4 which decrease its expression (27). However, thus far no evidence of natural cellular or humoral responses against CAIX have been reported.

Finally, it could be argued that if tumor immune responsiveness is pre-determined, non–immune-responsive tumors should not be treated. Obviously, this will depend on the mechanisms leading to immune responsiveness and whether they could be manipulated in vivo. This could be only determined when a better understanding of the algorithm determining immunologically mediated tumor rejection will be achieved. CAIX represents another important step in that direction.

References


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