

# The immunological tumor microenvironment predicts clinical outcome in human colorectal cancer

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## Abstract

In mice, tumor cells encountering host-immune defenses are vulnerable to host protection and tumor-shaping actions of the immune system. However, the relevance of natural anti-tumor immunity in man is still controversial. Infiltrating immune cells in tumors can account for variable outcomes, ranging from deleterious inflammatory processes to beneficial adaptative responses, but a protective role of infiltrating lymphocytes has been shown reported in several human tumors, such as, in melanoma and ovarian cancers. Colorectal cancer is one of the most common malignancies for both men and women, with an estimated annual incidence of 945,000 cases and 492,000 deaths worldwide.

We studied pathological signs of early metastatic invasion (venous emboli and lymphatic and perineural invasion) in 959 specimens of resected colorectal cancer. The local immune response within the tumor was studied by flow cytometry (39 tumors), low-density-array real-time polymerase-chain-reaction assay (75 tumors), and tissue microarrays (415 tumors).

As compared with tumors with signs of early metastatic invasion, tumors without such signs had increased infiltrates of immune cells and increased levels of messenger RNA (mRNA) for products of type 1 helper effector T cells (CD8, T-BET {T-box transcription factor 21}, interferon regulatory factor 1, interferon- $\gamma$ , granulysin, and granzyme B) but not increased levels of inflammatory mediators or immunosuppressive molecules. The two types of tumors had significant differences in the levels of expression of 65 combinations of T-cell markers, and hierarchical clustering showed that markers of T-cell migration, activation, and differentiation were increased in tumors without signs of early metastatic invasion. The latter type of tumors also had increased numbers of CD8+ T cells, ranging from early memory (CD45RO+CCR7-CD8+CD27+) to effector memory (CD45RO+CCR7-CD2BCD27-) T cells. The presence of high levels of infiltrating memory CD45RO+cells, evaluated immunohistochemically, correlated with the absence of signs of early metastatic invasion, a less advanced pathological stage, and increased survival.

Next, we characterized the tumor infiltrating immune cells in large cohorts of human colorectal cancers by gene expression profiling and by *in situ* immunohistochemical staining. Collectively, the immunological data (i.e., the type, density, and location of immune cells within the tumor samples) were found to be a better predictor of patient disease-free and overall survival than the histopathological methods currently used to stage colorectal cancer. These data support the hypothesis that the adaptative immune response influences the behavior of the human tumors both in terms of invasion, recurrence and survival.

## References

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