T-regulatory cells: key players in tumor immune escape and angiogenesis

T-regulatory cells (Tregs) are found infiltrating tumors in a vast array of tumor types, and tumor-infiltrating Tregs are often associated with a poor clinical outcome. Tregs are potent immunosuppressive cells of the immune system that promote progression of cancer through their ability to limit antitumor immunity and promote angiogenesis. Further, were highlighted several current therapies aimed at eliminating Tregs in cancer patients. Given the multifaceted role of Tregs in cancer, a greater understanding of their functions will ultimately strengthen future therapies.

Taken from: Facciabene A, Motz GT, Coukos G. T-Regulatory Cells: Key players in tumor immune escape and angiogenesis. Cancer Res. 2012;72(9):2162–71.

Vav3-rac1 signaling regulates prostate cancer metastasis with elevated vav3 expression correlating with prostate cancer progression and posttreatment recurrence

Prostate cancer remains the second leading cause of cancer death in men in the Western world. Yet, current therapies do not significantly improve the long-term survival of patients with distant metastases. The role of the guanine nucleotide exchange factor Vav3 in prostate cancer progression and metastasis was investigated and it was found that Vav3 expression correlated positively with prostate cancer cell migration and invasion. Stimulation of the receptor tyrosine kinase EphA2 by ephrinA1 resulted in recruitment and tyrosine phosphorylation of Vav3, leading to Rac1 activation as well as increased migration and invasion in vitro. Reduction of Vav3 resulted in fewer para-aortic lymph nodes and bone metastasis in vivo. Clinically, expression of Vav3 and EphA2 was elevated in late-stage and metastatic prostate cancers. Among patients with stage IIB or earlier prostate cancer, higher Vav3 expression correlated with lower cumulative biochemical failure-free survival, suggesting that Vav3 may represent a prognostic marker for posttreatment recurrence of prostate cancer. The findings provide evidence that the Vav3-mediated signaling pathway may serve as a therapeutic target for prostate cancer metastases.

Taken from: Lin KT, Gong J, Li CF, Jang TH, Chen WL, Chen HJ, et al. Vav3-rac1 signaling regulates prostate cancer metastasis with elevated vav3 expression correlating with prostate cancer progression and posttreatment recurrence. Cancer Res. 2012;72(12):3000-9.

Detection of tumor PIK3CA status in metastatic breast cancer using peripheral blood

DNA containing somatic mutations is highly tumor specific and thus can potentially be used as a biomarker. A novel technique for identifying cell free, plasma-derived circulating mutant DNA termed „BEAMing" has recently been developed. BEAMing is named after the 4 key components of the method (Beads, Emulsification, Amplification, and Magnetics). Assessment of circulating tumor DNA (ctDNA) by BEAMing can provide the mutational status of a patient's cancer. BEAMing can be carried out on virtually any tissue source without enriching for tumor cells, and thus the risk of „masking" mutations due to tumor heterogeneity and/or contamination of normal cells is greatly reduced. Use of plasma (peripheral blood) offers many advantages such as ease of access and the ability to repeat tests over time as the source of DNA is continuous. The overall frequency of PIK3CA mutations by BEAMing was similar in both patient cohorts (29% and 28.3%, respectively). In the retrospective cohort, the concordance of PIK3CA mutation status by BEAMing between formalin-fixed, paraffin-embedded (FFPE) samples and ctDNA from temporally matched plasma was 100% (34 of 34). In the prospective cohort, the concordance rate among 51 evaluable cases was 72.5% between BEAMing of ctDNA and sequencing of archival tumor tissue DNA. When the same archival tissue DNA was screened by both sequencing and BEAMing for PIK3CA mutations (n ¼ 41 tissue samples), there was 100% concordance in the obtained results.

Results suggest that the characterization of PIK3CA mutational status by testing a blood sample using BEAMing in patients with metastatic breast cancer is highly feasible. It was shown that BEAMing of plasma ctDNA

Table 1. Spectrum of *PIK3CA* mutations identified retrospectively in peripheral blood of patients with breast cancer by BEAMing of ctDNA and also by BEAMing of breast tumor tissue collected simultaneously from the same patients

Mutation	Amino acid change	Observed frequency in both ctDNA and tumor tissue/expected frequency[a] N = 49 (%)/(%)	Tumor hormone receptor positive N (%)	HER2 status of tumor N (%)	"Triple-negative" tumor
1633G>A	545E>K	3 (6.1)/(4.4)	3 (100)	2 (75) negative 1 (25) unknown	0
3140A>G	1047H>R	10 (20.4)/(13.3)	7 (70)	4 (40) positive 5 (50) negative 1 (10) unknown	1
3140A>T	1047H>L	1 (2)/(1.1)	1 (100)	1 (100) positive	0
Total number of mutations		14 (28.6)/(18.7)	11 (78.6)	5 (35.7) positive 7 (50) negative 2 (14.3) unknown	1 (7.1)

[a]Distribution of somatic mutations in PIK3CA, Catalogue Of Somatic Mutations in Cancer (COSMIC)

Table 2. Concordance of *PIK3CA* mutational status detected by sequencing or by BEAMing of DNA derived from the same tissue specimen (N = 41 matched samples, %)

Number of samples with adequate archival tissue available for BEAMing	41 (68.3)
Number of samples with adequate plasma available for ctDNA extraction and BEAMing	60 (100)
Number of samples that were *PIK3CA* wild-type by sequencing of archival tissue and *PIK3CA* wild-type by BEAMing of archival tissue	30 (73.1)
Number of samples that contained the same *PIK3CA* mutation by sequencing of archival tissue and by BEAMing of archival tissue	11 (26.8)
Number of discordant results between sequencing of archival tissue and BEAMing of archival tissue	0

correlates 100% with mutational status of a metastatic tumor specimen, when both samples are collected synchronously. If indeed PI3K inhibitors are shown to offer the greatest benefit in patients whose tumors harbor a PIK3CA mutation, the results suggest that the patients should optimally be selected for these trials based on PIK3CA mutational status at the time of enrollment, rather than on mutational status of archival tissue. BEAMing offers a reliable, noninvasive blood test to assess PIK3CA mutational status that could be theoretically conducted in lieu of a biopsy making it highly attractive to patients and health care providers.

Taken from: Higgins MJ, Jelovac D, Barnathan E, Blair B, Slater S, Powers P, et al. Detection of tumor PIK3CA status in metastatic breast cancer using peripheral blood. Clin Cancer Res. 2012;18(12): 3462–9.

Inhibiting systemic autophagy during Interleukin 2 immunotherapy promotes long-term tumor regression

Two decades ago, recombinant interleukin-2 (IL-2) received the U.S. Food and Drug Administration approval for the treatment of patients with advanced renal cancer and subsequently of patients with melanoma. High-dose IL-2 (HDIL-2) administration is associated with an objective 25% response rate in patients with kidney cancer, as reported in the recently completed IL-2 SELECT trial. Almost 20% of these patients survive more than 5 years. Attempts to improve the response rate and/or limit toxicity of IL-2 administration by inhibiting TNF, iNOS, or VEGF have failed. The greatest limitation of IL-2 treatment has been the associated side effects including hypotension as well as cardiac, gastrointestinal, renal, cerebral, pulmonary, and hepatic toxicity. These adverse effects are occasionally life threatening, and treatment is usually restricted to specialized centers, often resulting in early discontinuation or interruption of treatment. The precise mechanism mediating these side effects has not been clear. It was proposed that IL-2 toxicity is due to a cytokine-induced systemic autophagic syndrome. Recently, several cytokines including type II IFN and TGF-β have been shown to induce autophagy. The hypothesis was that the systemic syndrome associated with IL-2 treatment was related to cytokine-induced autophagy and temporally limited tissue dysfunction. The use of the autophagy inhibitor, chloroquine could limit toxicity and thereby enhance efficacy.

In vivo, chloroquine significantly inhibits 4T1 colorectal cancer growth and metastasis in murine models and induces apoptosis within the tumor microenvironment. Studies of human and murine cancer cell lines suggest that chloroquine may exert significant antitumor activity by inhibiting the induction of autophagy following cancer therapy. The hypothesis was that inhibition of autophagy with chloroquine in combination with HDIL-2 treatment would increase antitumor effects and promote survival when compared with IL-2 administration alone, enabling more effective expansion and function of immune cells.

Taken from: Liang X, De Vera ME, Buchser WJ, de Vivar Chavez AR, Loughran P, Beer Stolz D, et al. Inhibiting systemic autophagy during Interleukin 2 immunotherapy promotes long-term tumor regression. Cancer Res. 2012;72(11):2791–801.

STEAP proteins: from structure to applications in cancer therapy

The human 6-transmembrane epithelial antigen of prostate (STEAP) family comprises STEAP1, STEAP2, STEAP3, and STEAP4. All of these proteins are unique to mammals and share an innate activity as metalloreductases, indicating their importance in metal metabolism. Overall, they participate in a wide range of biologic processes, such as molecular trafficking in the endocytic and exocytic pathways and control of cell proliferation and apoptosis. STEAP1 and STEAP2 are overexpressed in several types of human cancers, namely prostate, bladder, colon, pancreas, ovary, testis, breast, cervix, and Ewing sarcoma, but their clinical significance and role in cancer cells are not clear. Still, their localization in the cell membrane and differential expression in normal and cancer tissues make STEAP proteins potential candidates as biomarkers of several cancers, as well as potential targets for new immunotherapeutic strategies for disease attenuation or treatment. This review brings together the current knowledge about each STEAP protein, giving an overview of the roles of this family of proteins in human physiology and disease, and analyzes their potential as immunotherapeutic agents in cancer research.

Taken from: Gomes IM, Maia CJ, Santos CR. STEAP Proteins: From Structure to Applications in Cancer Therapy. Mol Cancer Res. 2012;10(5):573-87.

Colorectal-cancer incidence and mortality with screening flexible sigmoidoscopy

Colorectal cancer is the second leading cause of cancer-related deaths in the United States. Colorectal-cancer mortality and incidence are reduced with screening by means of fecal occult-blood testing. Endoscopic screening with flexible sigmoidoscopy or colonoscopy is more sensitive than fecal testing for the detection of adenomatous polyps, the precursor lesions of colorectal cancer. Three European randomized trials of

Table 1. **Characteristics of the Study Participants.***

Characteristic	Flexible-Sigmoidoscopy Group (N=77,445)	Usual-Care Group (N=77,455)
	no. of participants (%)	
Sex		
Female	39,105 (50.5)	39,111 (50.5)
Male	38,340 (49.5)	38,344 (49.5)
Age		
55–59 yr	25,851 (33.4)	25,839 (33.4)
60–64 yr	23,783 (30.7)	23,771 (30.7)
65–69 yr	17,457 (22.5)	17,473 (22.6)
70–74 yr	10,354 (13.4)	10,372 (13.4)
Race or ethnic group†		
White (non-Hispanic)	66,874 (86.4)	65,708 (84.8)
Black (non-Hispanic)	3,883 (5.0)	3,825 (4.9)
Hispanic	1,421 (1.8)	1,397 (1.8)
Asian	2,791 (3.6)	2,785 (3.6)
Other or unknown	2,476 (3.2)	3,740 (4.8)
Educational level		
High-school graduate or less	22,892 (29.6)	22,583 (29.2)
Some college	25,935 (33.5)	25,585 (33.0)
College graduate	26,659 (34.4)	25,915 (33.5)
Unknown	1,959 (2.5)	3,372 (4.4)
Prior FOBT‡		
Yes	29,244 (37.8)	29,890 (38.6)§
No	43,858 (56.6)	42,223 (54.5)
Unknown	4,343 (5.6)	5,342 (6.9)
Prior lower GI endoscopy¶		
Yes	9,736 (12.6)	10,113 (13.1)§
No	64,653 (83.5)	62,997 (81.3)
Unknown	3,056 (3.9)	4,345 (5.6)
Either prior FOBT or prior lower GI endoscopy		
Yes	31,511 (40.7)	31,990 (41.3)§
No	40,648 (52.5)	39,161 (50.6)
Unknown	5,286 (6.8)	6,304 (8.1)
First-degree relative with colorectal cancer		
Yes	7,643 (9.9)	7,322 (9.5)
No	65,299 (84.3)	64,506 (83.3)
Unknown	4,503 (5.8)	5,627 (7.3)
Daily use of aspirin or ibuprofen in past 12 mo		
Yes	24,822 (32.1)	23,949 (30.9)‖
No	50,368 (65.0)	49,766 (64.3)
Unknown	2,255 (2.9)	3,740 (4.8)
Aspirin or ibuprofen use ≥3–4 times per wk in past 12 mo		
Yes	33,248 (42.9)	32,087 (41.4)**
No	41,971 (54.2)	41,658 (53.8)
Unknown	2,226 (2.9)	3,710 (4.8)

* There were no significant differences between the groups except as noted. FOBT denotes fecal occult-blood test, and GI gastrointestinal.
† Race or ethnic group was determined by self-report.
‡ Prior FOBT indicates a test within 3 years before study entry.
§ P<0.001
¶ Prior lower GI endoscopy indicates sigmoidoscopy, colonoscopy, or barium enema examination within 3 years before randomization.
‖ P=0.03
** P=0.01

flexible sigmoidoscopy have been performed. In the United Kingdom, a one-time screening with flexible sigmoidoscopy significantly reduced the incidence of colorectal cancer (by 23%) and associated mortality (by 31%). In Italy, an 18% reduction in incidence and a nonsignificant 22% reduction in mortality were observed, whereas in Norway, no benefit was observed after 7 years of follow-up.

From 1993 through 2001, we randomly assigned 154,900 men and women of 55 to 74 years of age either to screening with flexible sigmoidoscopy, with a repeat screening at 3 or 5 years, or to usual care. Cases of colorectal cancer and deaths from the disease were ascertained.

Of the 77,445 participants randomly assigned to screening (intervention group), 83.5% underwent baseline flexible sigmoidoscopy and 54.0% were screened at 3 or 5 years. The incidence of colorectal cancer after a median follow-up of 11.9 years was 11.9 cases per 10,000 person-years in the intervention group (1012 cases), as compared with 15.2 cases per 10,000 person-years in the usual-care group (1287 cases), which represents a 21% reduction (relative risk, 0.79; 95% confidence interval [CI], 0.72 to 0.85; P<0.001). Significant reductions were observed in the incidence of both distal colorectal cancer (479 cases in the intervention group vs. 669 cases in the usual-care group; relative risk, 0.71; 95% CI, 0.64 to 0.80; P<0.001) and proximal colorectal cancer (512 cases vs. 595 cases; relative risk, 0.86; 95% CI, 0.76 to 0.97; P = 0.01). There were 2.9 deaths from colorectal cancer per 10,000 person-years in the intervention group (252 deaths), as compared with 3.9 per 10,000 person-years in the usual-care group (341 deaths), which represents a 26% reduction (relative risk, 0.74; 95% CI, 0.63 to 0.87; P<0.001). Mortality from distal colorectal cancer was reduced by 50% (87 deaths in the intervention group vs. 175 in the usual-care group; relative risk, 0.50; 95% CI, 0.38 to 0.64; P<0.001); mortality from proximal colorectal cancer was unaffected (143 and 147 deaths, respectively; relative risk, 0.97; 95% CI, 0.77 to 1.22; P = 0.81).

Screening with flexible sigmoidoscopy was associated with a significant decrease in colorectal-cancer incidence (in both distal and proximal colon) and mortality (distal colon only). (Funded by the National Cancer Institute; PLCO

Table 2. **Colorectal-Cancer Incidence and Mortality.***

Variable	Flexible-Sigmoidoscopy Group (N=77,445)		Usual-Care Group (N=77,455)		Relative Risk (95% CI)	P Value
	no. of participants	*rate per 10,000 person-yr (95% CI)*	*no. of participants*	*rate per 10,000 person-yr (95% CI)*		
Incidence						
All colorectal cancers	1012	11.9 (11.2–12.7)	1287	15.2 (14.4–16.0)	0.79 (0.72–0.85)	<0.001
Location of cancer†						
Distal	479	5.6 (5.1–6.2)	669	7.9 (7.3–8.5)	0.71 (0.64–0.80)	<0.001
Proximal	512	6.0 (5.5–6.6)	595	7.0 (6.5–7.6)	0.86 (0.76–0.97)	0.01
Sex						
Male	567	13.6 (12.4–14.7)	768	18.5 (17.2–19.9)	0.73 (0.66–0.82)	<0.001
Female	445	10.3 (9.4–11.3)	519	12.0 (11.0–13.0)	0.86 (0.76–0.98)	0.02
Age at randomization						
55–64 yr	518	9.4 (8.6–10.2)	662	12.1 (11.2–13.0)	0.78 (0.69–0.87)	<0.001
65–74 yr	494	16.6 (15.1–18.1)	625	20.9 (19.3–22.5)	0.79 (0.71–0.89)	<0.001
Mortality						
All colorectal-cancer deaths	252	2.9 (2.5–3.2)	341	3.9 (3.5–4.3)	0.74 (0.63–0.87)	<0.001
Location of cancer†						
Distal	87	1.0 (0.8–1.2)	175	2.0 (1.7–2.3)	0.50 (0.38–0.64)	<0.001
Proximal	143	1.6 (1.4–1.9)	147	1.7 (1.4–2.0)	0.97 (0.77–1.22)	0.81
Sex						
Male	139	3.2 (2.7–3.8)	211	4.9 (4.3–5.6)	0.66 (0.53–0.81)	<0.001
Female	113	2.6 (2.1–3.0)	130	2.9 (2.4–3.4)	0.87 (0.68–1.12)	0.28
Age at randomization						
55–64 yr	133	2.4 (2.0–2.8)	157	2.8 (2.3–3.2)	0.84 (0.67–1.06)	0.16
65–74 yr	119	3.9 (3.2–4.6)	184	6.0 (5.1–6.9)	0.65 (0.52–0.82)	<0.001

* The median follow-up time for incidence was 11.9 years (interquartile range, 10.2 to 13.0) and for mortality was 12.1 years (interquartile range, 10.4 to 13.0).
† Distal location was defined as the rectum through the splenic flexure, and proximal as the transverse colon through the cecum. For incidence, the location was unknown for 21 cases in the flexible-sigmoidoscopy group and 23 cases in the usual-care group. For mortality, the location was unknown for 22 deaths in the flexible-sigmoidoscopy group and 19 deaths in the usual-care group.

ClinicalTrials.gov number, NCT00002540.) A total of 77,445 participants were randomly assigned to flexible sigmoidoscopy, and 77,455 to usual care. The baseline characteristics of the participants were similar in the two study groups; the median follow-up time was 11.9 years, and the mean follow-up time was 11.0 years. The incidence of colorectal cancer was 11.9 cases per 10,000 person-years in the intervention group (1012 cases), as compared with 15.2 cases per 10,000 person-years in the usual-care group (1287 cases), which represents a 21% reduction (relative risk, 0.79; 95% confidence interval [CI], 0.72 to 0.85; P<0.001). Significant reductions were observed in the incidence of both distal colorectal cancer (479 cases in the intervention group vs. 669 cases in the usual-care group; relative risk, 0.71; 95% CI, 0.64 to 0.80; P<0.001) and proximal colorectal cancer (512 cases vs. 595 cases; relative risk, 0.86; 95% CI, 0.76 to 0.97; P = 0.01).

Taken from: Schoen RE, Pinsky PF, Weissfeld JL, Yokochi LA,Church T, Laiyemo AO, et al. Colorectal-cancer incidence and mortality with screening flexible sigmoidoscopy. N Engl J Med. 2012;366:2345-57.