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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Studies relating to the role autophagy in tumor dormancy revealed that transient inhibition of autophagy during ADR treatment resulted in prolonging tumor dormancy. However, complete knockdown of autophagy gene expedited tumor relapse. The purpose of the study was to determine if transient blockade of autophagy by CQ was associated with anti-inflammatory function of CQ independent of its role in autophagy. We showed that inhibition of tumor-intrinsic inflammatory pathways, which was induced by ADR, prolonged tumor dormancy, in vitro and in vivo. Also, induction of tumor-intrinsic inflammatory pathways reduced immune modulatory function of chemotherapy on dormant tumor cells, while increasing their susceptibility to anti-tumor T cell responses. These data suggests a paradoxical role for ADR-induced tumor-intrinsic inflammatory pathways, facilitating tumor relapse on the one hand and increasing susceptibility of dormant tumor cells to immunotherapy on the other hand. Therefore, immunotherapy could be an option for preventing tumor relapse following chemotherapy-induced tumor dormancy.

15. SUBJECT TERMS
Autophagy, tumor dormancy, tumor relapse, chemotherapy, immunotherapy

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1. **INTRODUCTION:**
   The objective of the proposal is to understand the role of autophagy in chemotherapy induced tumor dormancy and recurrence.

2. **KEYWORDS:**

   tumor dormancy, tumor relapse, immunotherapy, immunoediting, autophagy

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

1) Understand the role of autophagy in chemotherapy-induced tumor dormancy (Aim 1)
2) Understand the role of tumor IFN-gamma Ra in determining tumor recurrence under immune pressure (Aim 2)

What was accomplished under these goals?

Since autophagy was not a major player in tumor dormancy or relapse (Progress Report, Year 2), we explored anti-inflammatory function of CQ in prolonging tumor dormancy, in vitro. The results presented below are included in a manuscript that is in preparation. We plan to submit the manuscript by the end of June 2017.

Dormant and relapsing MMC show unique inflammatory signature

We performed microarray analysis for each experimental group (n=3). Untreated MMC and MMC+CQ day 4 were included as controls. Fold change in gene expression for each group was determined by comparison with untreated MMC control. Unsupervised cluster analysis shows close clustering among the ADR+CQ groups at both the 3-week and 6-week time points, representing maintenance of dormancy. Conversely, the ADR groups at the 3-week and 6-week time points, representing dormancy and relapse, clustered apart from one another (Fig. 1). We then sought to determine the gene profile unique to each experimental group. Venn diagram analysis showed 239 genes unique to dormancy when both dormant groups were compared (MMC+ADR week 3 vs MMC+ADR+CQ week 3) (Fig. 2A upper panel). 682 genes unique to prolonged dormancy were found by comparing the fold changes of the relapsing group (MMC+ADR week 6) with the prolonged dormancy group (MMC+ADR+CQ week 6) (Fig. 2A lower panel). The prolonged dormancy group (MMC+ADR+CQ week 6) and relapsing group (MMC+ADR week 6) shared 882 common probe sets. Each probe set group was then analyzed for disease function by Ingenuity Pathway Analysis (IPA). The 239 genes involved in dormancy showed a z-score increase in disease states related to acute inflammation, while the 682 genes unique to relapse showed predicted activation in disease states related to chronic inflammation (Fig. 2B). The 882 genes shared by both week 6 groups, one relapsing and the other dormant, showed predicted activation of both chronic and acute disease states.

In order to pinpoint pathways and proteins involved in inflammation, all probe sets that showed a significant upregulation or downregulation for each experimental group were uploaded to IPA and comparison analysis on canonical pathways and upstream regulators was performed (Fig. 2C). Most notably, RelA (NF-κB p65) showed predicted activation (z-score=2.6) only in the MMC+ADR week 3 dormant group. NF-κB (complex) showed predicted activation in all groups, however, the highest z-scores were in the MMC+ADR week 6 relapsing group and MMC+ADR+CQ week 6 prolonged dormancy group (z-score=3.26 and 2.79). Type 1 interferons (alpha and beta) showed predicated activation in the MMC+ADR week 3 dormant group (z-score= 3.5 and 2.6) and the MMC+ADR+CQ week 3 dormant group (z-score=2.5 and 1.77). Interferon gamma (IFNG) also showed predicted activation in both dormancy groups (z-

score=2.9 and 2.5). Interferon Signaling was shown to be highly activated (z-score=3.16) only in the MMC+ADR week 3 dormant group.

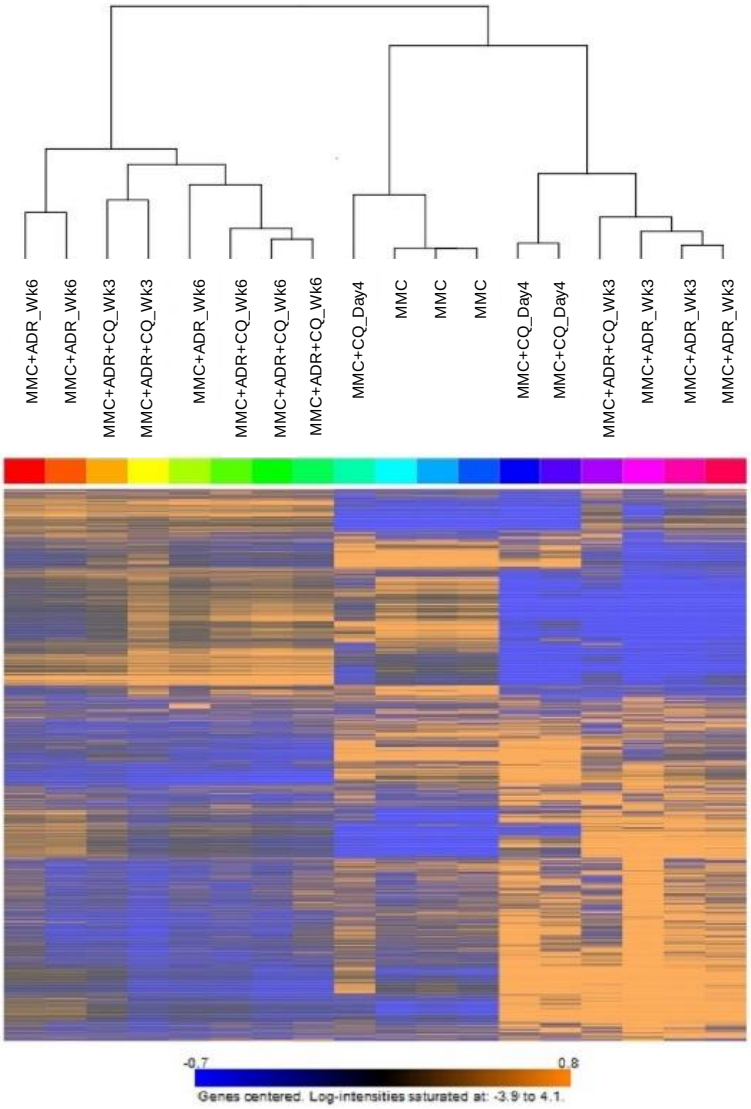

Fig. 1. Dormant MMC exhibit unique gene signature compared to relapsing MMC

Unsupervised cluster analysis shows a close clustering of experimental groups MMC+ADR+CQ at weeks 3 and 6 during the establishment and maintenance of tumor dormancy. However, tumor cells that enter the dormant state at week 3 and resumed a sluggish proliferation at week 6 (MMC+ADR week 3 and week 6) were clustered apart from each other. Control groups (MMC and MMC+CQ) were clustered together.

In order to determine whether the predicted activation of RelA led to the secretion of inflammatory cytokines during dormancy (preceding relapse) a multiplex cytokine array was performed on the supernatant from the MMC+ADR week 3 group. Cytokines probes were chosen by analysis of robust multi-array average (RMA) expression data for inflammatory cytokines that showed unique upregulation during dormancy. Choice of cytokine was also limited based on market availability. The protein concentration corroborated mRNA expression level from the microarray data for each cytokine, except RANTES, which shows a decrease in protein expression (Fig. 3).

ADR-treated shNF-κB p65 exhibit reduced growth and a reduced rate of relapse *in vitro* and *in vivo.*

Though CQ is most noted for its effects on blocking autophagy, unpublished data from our lab showed that autophagy protein 5 (ATG5) shRNA knockdown MMC resulted in a higher rate of relapse compared to control MMC. Therefore, transient blockade of autophagy alone during drug treatment could not be the cause for the delay in relapse of that group. In addition to blockade of autophagy, CQ has been shown to inhibit NF-κB through blockade of IkB degradation (1). Because of such findings, and IPA results

suggesting unique NF-κB p65 signaling pathways during dormancy, we created an shRNA knockdown of NF-κB p65 in MMC in order to determine if prolonged dormancy in CQ-treated MMC was due to NF-κB inhibition. MMC were transduced using lentiviral particles containing NF-κB p65 shRNA (shNF-κB p65) or SCR shRNA (SCR-MMC) and remaining cells were subject to puromycin selection and western blot analysis (Fig. 4A). Both groups showed similar rates of proliferation, in vitro (Fig. 4B).

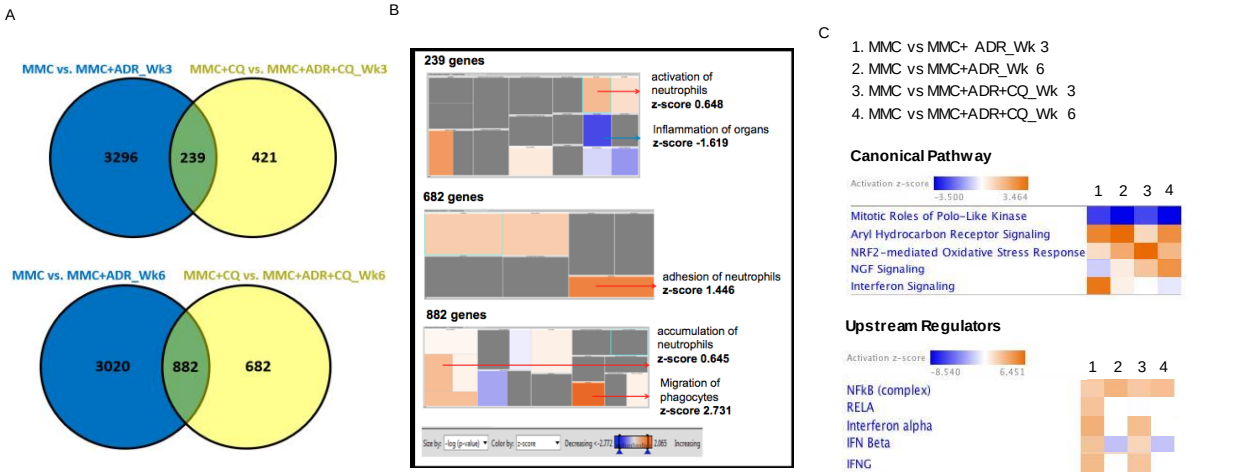

Fig. 2. Inflammatory gene signature during dormancy and relapse. A) Venn Diagram analysis isolated 239 probe sets unique to dormancy when comparing both dormant groups (upper panel) and 682 unique probe sets unique to relapse when both week 6 groups were compared (lower panel). 882 probe sets were shared between the MMC+ADR week 6 relapsing group and the MMC+ADR+CQ week 6 prolonged dormancy group. Venn diagrams were generated from microarray fold-change expression data. Fold change was calculated by comparing each group to MMC control expression. B) Ingenuity Pathway Analysis (IPA) reveals genes among 239 shared probe sets involved in maintenance of dormancy shows predicted activation of disease states related to acute inflammation, 682 probe sets unique to relapse show predicated activation of disease states related to acute inflammation, and 882 shared probe sets involved in both maintenance and escape from dormancy show predicated activation of disease states related to acute and chronic inflammation. C) IPA comparison analysis shows predicted activation and inhibition of canonical pathways and upstream regulators based on z-score. RelA showed a significant (z-score=2.6) predicted activation in the dormant group (MMC+ADR week 3). All significant ($p<0.0001$) fold change expression data for each experimental group was included in comparison analysis.

Growth rate and response to ADR treatment was determined *in vivo* by subcutaneously injecting FVBN202 mice with 3 million shNF-κB p65 or SCR-MMC in the mammary fat pad (n=3). Tumor size initially showed no significant changes in growth between both groups (Fig. 5A). However, when animals were treated intravenously with ADR (9mg/kg) every 3 days beginning on day 36, the shNF-κB p65 tumors showed significantly ($p=0.01$) reduced growth compared to SCR-MMC tumors by day 54. Fractions of tumor cells were then cultured *in vitro* for 2 weeks upon resection from the animal in order to confirm stable shRNA knockdown of NF-κB p65. Western blot analysis of each tumor shows maintained knock down of NF-κB p65 to varying degrees (Fig. 4B).

The shNF-κB p65 MMC showed increased neu expression in response to ADR treatment *in vitro* and *in vivo*

We then sought to determine if tumor-intrinsic NF-κB p65 signaling pathways had any effect on immunomodulation of ADR-treated MMC by analyzing neu, PD-L1, and MHCI expression. shNF-κB p65 and SCR-MMC were treated with ADR as described above (n=3). On day 7 post treatment, cells were detached and analyzed for neu, PD-L1, and MHCI expression by flow cytometry. Mean florescence intensity (MFI) showed significant upregulation of neu expression in ADR-treated cells when compared to untreated control in both groups ($p=0.0007$ and $p=0.01$) (Fig. 6A). However, shNF-κB p65 displayed significantly higher upregulation when compared to SCR-MMC ($p=0.006$). Both shNF-κB p65 and SCR-MMC showed no increase in PD-L1 MFI after ADR treatment, and while MHCI MFI did increase upon ADR treatment, there were no significant differences between shNF-κB p65 and SCR-MMC. Due to significant changes in neu expression between ADR-treated shNF-κB p65 and SCR-MMC, we chose to focus solely on

neu expression for *in vivo* staining. Tumors resected from shNF-κB p65 or SCR-MMC-inoculated mice treated with ADR (n=3) (described above), along with control mice (n=1), were stained for neu expression following the same protocol. Neu upregulation showed the same trend *in vivo*, with increased neu MFI in ADR-treated mice but a larger increase in the shNF-κB p65 compared to SCR-MMC tumors (p=<0.0001) (Fig. 6B).

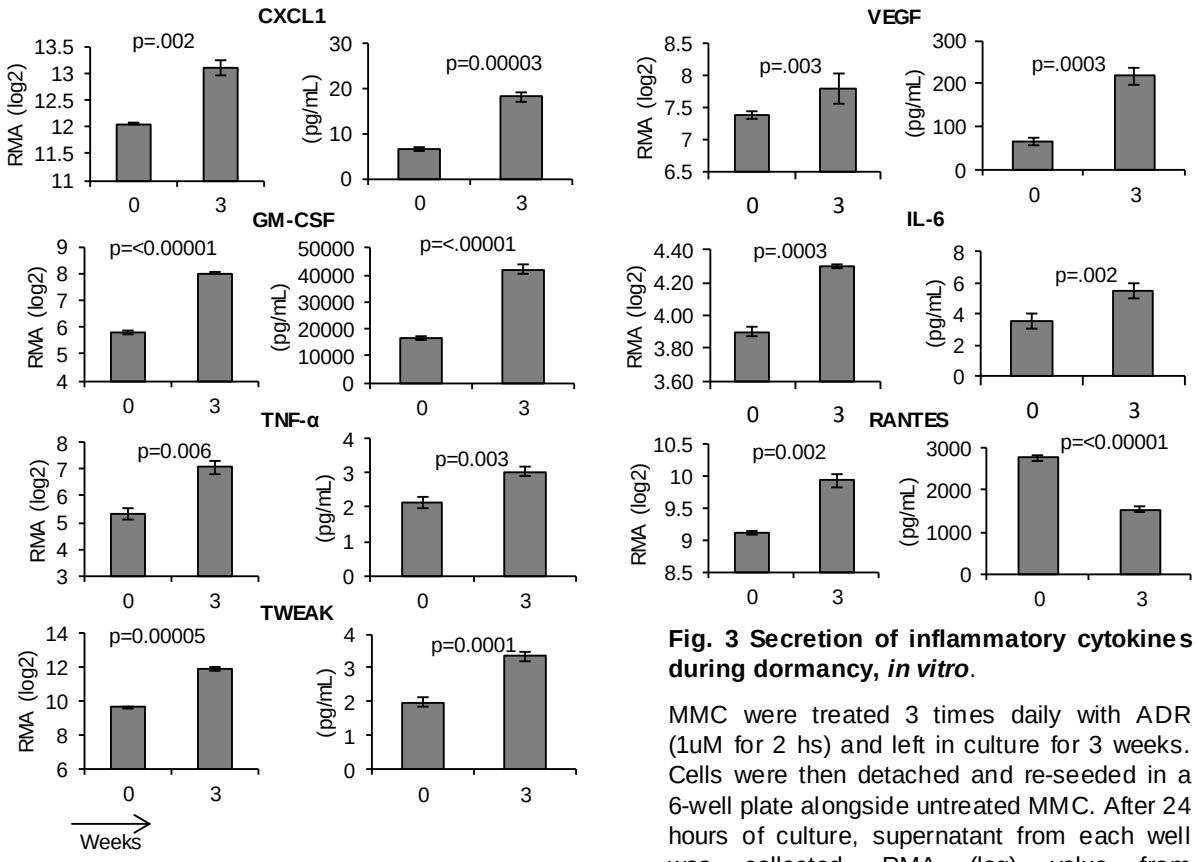

Fig. 3 Secretion of inflammatory cytokines during dormancy, *in vitro*.

MMC were treated 3 times daily with ADR (1uM for 2 hs) and left in culture for 3 weeks. Cells were then detached and re-seeded in a 6-well plate alongside untreated MMC. After 24 hours of culture, supernatant from each well was collected. RMA (log) value from microarray data and concentration (pg/mL) of each cytokine are compared side-by-side. Targets were chosen based on microarray data and commercial availability.

The shNF-κB p65 MMC show reduced tumor-infiltrate and a reduced immunostimulatory effects

We then sought to determine if tumor-intrinsic NF-κB p65 signaling pathways had an effect on infiltration of CD45+ immune cells or the particular immune-cell type. shNF-κB p65 or SCR-MMC tumors resected from ADR-treated mice (n=3) were stained with CD45 and compared with that of non-treated mice (n=1) (Fig. 7A). Additional staining for CD11b, GR1, CD3, CD4, CD8, B220, CD49b was performed only on non-treated mice from each group. Log_2 frequency ratios showed the fold change increase or decrease in percentages of each cell type and was calculated by comparing the log_2 ratio of shNF-κB p65 sample percentages to those of SCR-MM (2). While total CD45+ infiltrate showed no significant differences between the ADR-treated groups, the ADR-treated wild type tumors (Scr-MMC) showed greater CD45+ infiltrates than their untreated control cells, when compared with the shNF-κB p65 MMC tumor cells. The Scr-MMC tumor site contained an increased CD11b+GR1+ MDSCs compared to the SCR-MMC tumor. In addition, the shNF-κB p65 MMC tumors showed a decreased CD8+ T cell infiltrates.

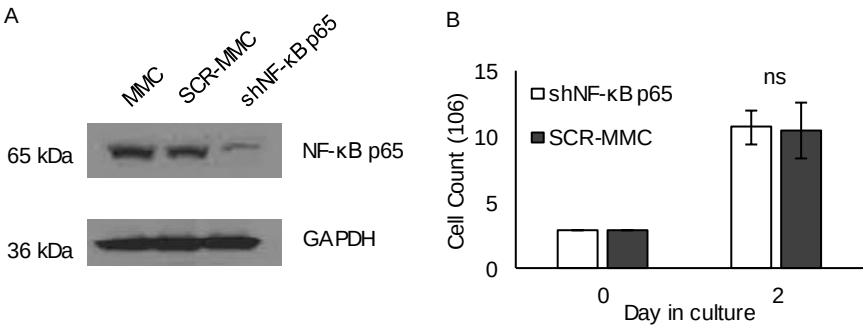

Fig. 4 NF-κB p65 knockdown in MMC reduces the rate of relapse, *in vitro*. A) Western Blot analysis shows knock down of NF-κB p65 shRNA-transduced MMC, after puromycin selection, alongside control MMC and SCR-MMC. GAPDH was used as the housekeeping control. B) shNF-κB p65 and SCR-MMC were seeded at 3 x 10^6 cells/flask and left in culture for 48 hours. Cells were detached and counted via trypan blue exclusion. Data represent 3 independent experiments and mean ± SEM.

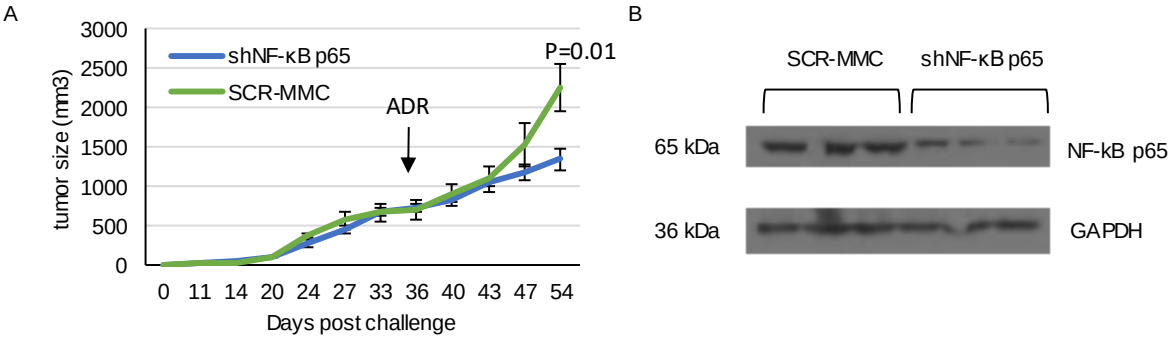

Fig. 5. The shNF-κB p65 tumors show reduced growth in response to ADR treatment, in vivo . A) 3 x 10^6 shNF-κB p65 or SCR-MMC were injected subcutaneously into the mammary fat pad of FVBN202 naïve mice (n=3). Mice were treated every 3 days with 9mg/kg of ADR beginning 36 days post challenge, when tumors has reached 800mm^3, and euthanized on day 54 when tumors had reached greater than 2000mm^3. Tumors were measured twice weekly by digital caliper. B) Remaining tumor samples were cultured for two weeks and western blot analysis was performed separately for each animal.

In order to investigate the role of NF-κB p65 in the anti-tumor immune response, splenocytes were collected from ADR-treated mice which had been inoculated with either shNF- κB p65 or SCR-MMC tumors (n=3). Reprogramming of tumor-sensitized immune cells was done *ex vivo*, as previously described by our group (3). Splenocytes from shNF-κB p65 tumors showed significant (p=0.05) reduction in expansion, based on final cell count, compared with those from SCR-MMC tumor mice (Fig. 7B). MMC remained sensitive to tumor-reactive lymphocytes taken from SCR-MMC tumor mice, with 65% apoptosis of target MMC. However, tumor-sensitized lymphocytes isolated from shNF-κB p65 tumor mice showed reduced cytotoxic function against MMC, inducing 49% apoptosis (p=0.05).

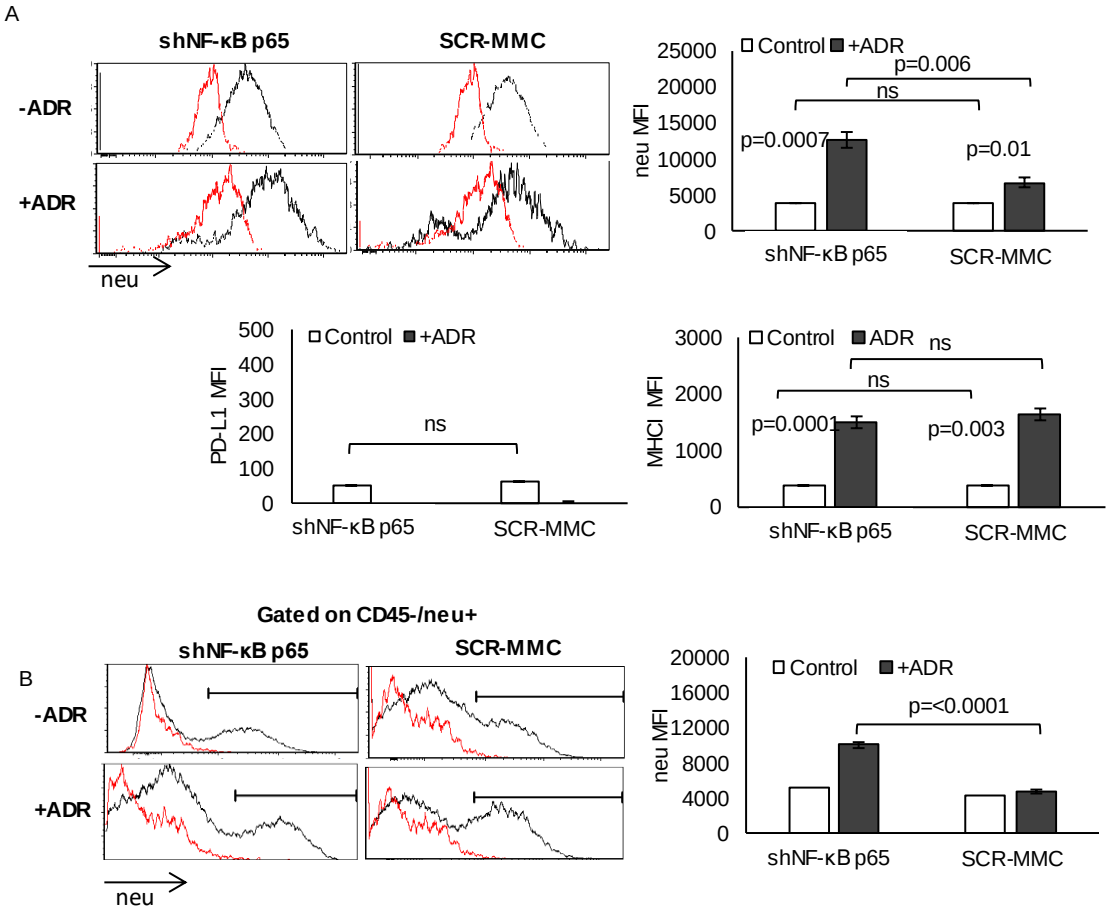

Fig. 6. The shNF-κB p65 MMC show higher upregulation of neu in response to ADR treatment. A) shNF-κB p65 and SCR-MMC were treated 3 times daily with ADR (1uM for 2 hrs) and left in culture for 1 week. Neu, PD-L1, and MHC1 expression was determined by MFI B) 3 x 10^6 shNF-κB p65 or SCR-MMC were injected subcutaneously into the mammary fat pad of FVBN202 naïve mice. Mice were treated every 3 days with 9mg/kg ADR 36 days post challenge, when tumors has reached 800mm^3, and euthanized on day 54 when tumors had reached greater than 2000mm^3. Control mice were injected with 5 x 10^6 of each and euthanized when tumors had reached >800mm^3. Tumors were resected from mice and measured for neu expression by flow cytometry. MFI was determined by gating on CD45-/neu+ cells only.

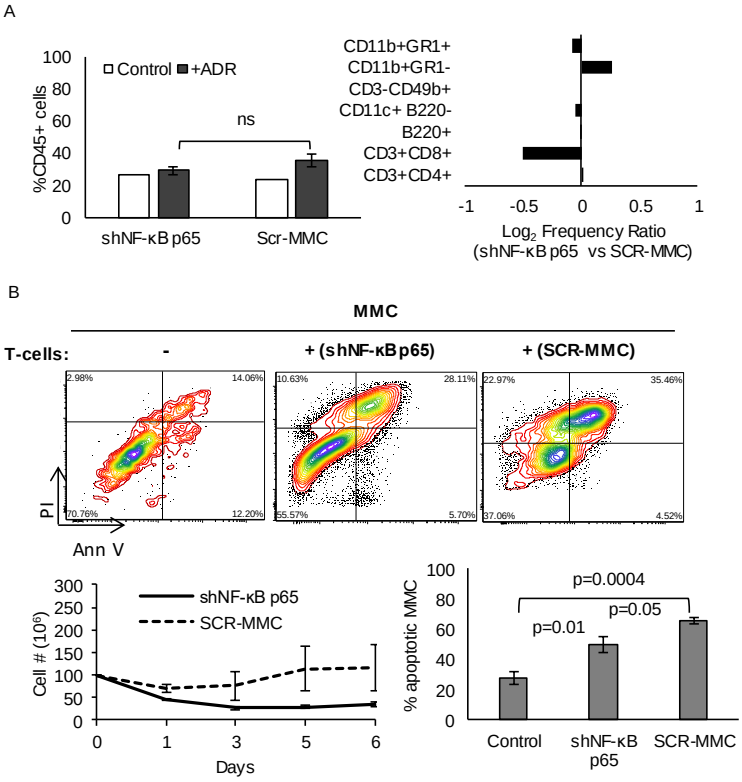

Fig. 7. The shNF-κB p65 tumors display alteration of tumor infiltrate in vivo and T-cell activation ex vivo. A) 3 x 10^6 shNF-κB p65 or SCR-MMC were injected subcutaneously into the mammary fat pad of FVBN202 naïve mice (n=3). Mice were treated every 3 days with 9mg/kg ADR 36 days post challenge when tumors has reached 800mm^3. Mice were euthanized on day 56 when tumors had reached greater than 2000mm^3. Control mice were injected with 5 x 10^6 shNF-κB p65 or SCR-MMC and euthanized when tumor reached 1000mm^3. Tumors were resected from ADR-treated mice and analyzed for CD45 expression. Control mice tumors were analyzed for CD45, B220, CD3, CD8, CD4, CD11b, CD11c, GR1 and CD49b expression. B) For cytotoxicity assay, MMC were cultured with expanded T-cells from splenocytes of shNF-κB p65 or SCR-MMC inoculated mice and cultured for two days in the presence of IL-2. *Ex vivo* expansion prior to co-culture shows poor expansion of splenocytes from shNF-κB p65 mice (bottom left). In cytotoxicity assay, apoptotic MMC were determined by Annexin V+ and PI+ cells. Representative dot plots show cells in early/late apoptosis and necrosis (top). Total apoptotic includes early apoptotic, late apoptotic and necrotic cells (bottom right).

References

1. Jia L, Gopinathan G, Sukumar JT, Gribben JG. Blocking autophagy prevents bortezomib-induced NF-kappaB activation by reducing I-kappaBalpha degradation in lymphoma cells. PLoS One 2012;7:e32584.

2. Pachynski RK, Scholz A, Monnier J, Butcher EC, Zabel BA. Evaluation of tumor-infiltrating leukocyte subsets in a subcutaneous tumor model. J Vis Exp 2015;(98). doi:10.3791/52657.

3. Kmieciak M, Basu D, Payne KK, Toor A, Yacoub A, Wang XY, et al. Activated NKT cells and NK cells render T cells resistant to myeloid-derived suppressor cells and result in an effective adoptive cellular therapy against breast cancer in the FVBN202 transgenic mouse. J Immunol 2011;187:708-17.

What opportunities for training and professional development has the project provided?

- Two PhD students, *Bryan McKiver* and *Timothy M Smith*, did their rotation in the laboratory of Dr. Manjili. *Timothy Smith* joined the lab to conduct his PhD related to tumor dormancy and immunotherapy.
- Two medical students, *John Laurenzano* and *Stephen Keffer*, conducted their summer research program in the laboratory of Dr. Manjili.
- One medical school candidate, *Melika Zarei*, did her volunteer work on this project in the laboratory of Dr. Manjili. She was the admitted to Virginia Tech Carilion School of Medicine, Roanoke, VA.
- A visiting scientist, *Ying Han*, MD, from Tongii Hospital, Tongii Medical College, Wuhan, China worked on this project during her training in the area of tumor dormancy.

How were the results disseminated to communities of interest?

1) Concepts that are proposed in this project were used to formulate two graduate level lectures- advanced immunology and molecular biology of cancer- related to cancer dormancy.
2) As an invited speaker, Dr. Manjili gave a lecture on tumor dormancy in Mayo Clinic, Rochester MN. Title: *Immunotherapy is the only option for the treatment of cancer dormancy*". July 2017
3) As an invited speaker, Dr. Manjili gave a lecture on tumor dormancy in The VCU Institute of Molecular Medicine (VIMM) and Human & Molecular Genetics (HMG) seminar, VCU School of Medicine, Richmond VA. Title: *Paradigm shifts in cancer immunotherapy: targeting tumor dormancy.* May 2017
4) Two posters were presented at IMMUNOLOGY 2017™ AAI Annual Meeting, Washington D.C., May 12-16, 2017.

What do you plan to do during the next reporting period to accomplish the goals?

Our request for NCE was approved in order to complete the studies related to IFN-g Ra. We will perform T cell depletion studies in vivo in order to determine whether immunogenic tumor dormancy and the host immune response are responsible for prolonging tumor dormancy. We will also perform studies to determine the contribution of the tumor IFN-g Ra in tumor dormancy or recurrence.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Our paper from this project was the most read article over the last six months of 2016 in Journal of Leukocyte Biology. First author of the manuscript, Kyle Payne, was invited to speak at the 2017 Annual Meeting of the Society for Leukocyte Biology (SLB) to be held in Toronto, October 5-7, 2017.

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Papers

1. **Manjili MH**. Tumor dormancy and relapse: from a natural by-product of evolution to a disease state. *Cancer Res* 77 (10) 2564-2569, 2017
2. **Manjili MH** and Payne KK. Immune regulatory function of Tregs. *Immunol Invest.* 45(8):708-711, 2016. PMID: 27775448
3. **Manjili MH** and Butler SE. Role of Tregs in cancer dormancy or recurrence. *Immunol Invest* 45(8):759-766, 2016 PMID: 27603507

Presentations (invited speaker):

1) Invited speaker, Mayo Clinic, Rochester MN. Title: *Immunotherapy is the only option for the treatment of cancer dormancy*", July 2016
2) Invited speaker, The VCU Institute of Molecular Medicine (VIMM) and Human & Molecular Genetics (HMG) seminar, VCU School of Medicine, Richmond VA. Title: *Paradigm shifts in cancer immunotherapy: targeting tumor dormancy*, May 2017

- **Website(s) or other Internet site(s)**

- Research Report, VCU Massey Cancer Center (March 2017): Manjili's study most read article over the last six months in JLB: http://myemail.constantcontact.com/Massey-Research-Report-for-March-2017.html?soid=1101948063140&aid=H9ev46PEC3Y
- **"**New combination of chemotherapy and immunotherapy combats breast cancer cell recurrence", Massey Cancer center Achieve, 12/08/2016, https://massey.vcu.edu/news/blog/2016/new-combination-of-chemotherapy-and-immunotherapy-combats-breast-cancer-cell-recurrence/
- Combination chemotherapy-immunotherapy may help eliminate dormant tumor cells. Oncology Central 09/12/2016. http://www.oncology-central.com/2016/09/12/combination-immunotherapy-chemotherapy-may-help-eliminate-dormant-tumor-cells/
- Step toward eliminating cancer recurrence. Next Big Future 09/02/2016: http://www.nextbigfuture.com/2016/09/step-toward-eliminating-cancer.html?utm_source=feedburner&utm_medium=feed&utm_campaign=Feed%3A+blogspot%2Fadvancednano+%28nextbigfuture%29

- Cancer relapse risk reduced by combining chemotherapy and immunotherapy. Medical Daily 09/02/2016: http://www.medicaldaily.com/combination-chemotherapy-and-immunotherapy-reduce-cancer-relapse-study-says-396900
- "Researchers take step toward eliminating cancer recurrence" September 1, 2016
- Eurekalert: http://www.eurekalert.org/pub_releases/2016-09/foas-rts090116.php
- ScienceDaily: https://www.sciencedaily.com/releases/2016/09/160901125047.htm
- Medicalxpress: http://medicalxpress.com/news/2016-09-cancer-recurrence.html
- "Immunotherapy Plus Chemotherapy Kills More Cancer Cells Than Chemotherapy Alone" J Clinical Pathways, September 9, 2016 http://www.journalofclinicalpathways.com/article/immunotherapy-plus-chemotherapy-kills-more-cancer-cells-chemotherapy-alone

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Savannah Butler
Project Role:	Lab specialist/graduate student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	Ms. Butler has performed in vitro and in vivo studies of chemotherapy-induced tumor dormancy
Funding Support:	DoD, MCC Pilot Project Award
Name:	Timothy Smith, PhD
Project Role:	Graduate student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	Mr. Smith has performed in vitro studies of chemotherapy-induced tumor dormancy.
Funding Support:	DoD, PhD scholarship
Name:	Hussein Aqbi
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	9

Contribution to Project:	Mr. Aqbi has performed in vivo studies associated with chemotherapy-induced tumor dormancy, and immune response studies.
Funding Support:	DoD, PhD scholarship
Name:	Ying Han
Project Role:	Visiting Fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	Dr. Han has performed in vitro studies of breast cancer dormancy.
Funding Support:	DoD, fellowship award

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS:

COLLABORATIVE AWARDS: The partnering PI, Dr. David Gewirtz, is submitting an independent progress report

9. APPENDICES: Documents attached

APPENDIX

Review

Cancer Research

Tumor Dormancy and Relapse: From a Natural Byproduct of Evolution to a Disease State

Masoud H. Manjili

Abstract

Species evolve by mutations and epigenetic changes acting on individuals in a population; tumors evolve by similar mechanisms at a cellular level in a tissue. This article reviews growing evidence about tumor dormancy and suggests that (i) cellular malignancy is a natural byproduct of evolutionary mechanisms, such as gene mutations and epigenetic modifications, which is manifested in the form of tumor dormancy in healthy individuals as well as in cancer survivors; (ii) cancer metastasis could be an early dissemination event that could occur during malignant dormancy even before primary cancer is clinically detectable; and (iii) chronic inflammation is a key factor in awakening dormant malignant cells at the primary site, leading to primary cancer development, and at distant sites, leading to advanced stage diseases. On the basis of this evidence, it is reasonable to propose that we are all cancer survivors rather than cancer-free individuals because of harboring dormant malignant cells in our organs. A better understanding of local and metastatic tumor dormancy could lead to novel cancer therapeutics for the prevention of cancer. *Cancer Res; 77(10); 2564–9. ©2017 AACR.*

Malignancy Is a Byproduct of Evolutionary Mechanisms of Cell Survival

DNA is a dynamic and adaptable molecule that is constantly changing through the process of mutation and epigenetic modification. These are evolutionary mechanisms that allow survival of an individual against environmental insults. DNA mutation could spontaneously occur during DNA replication or could be accidental as a result of environmental exposure to certain chemicals, UV radiation, or other external factors that impact DNA replication. Spontaneous somatic mutations lead to genotypic and phenotypic heterogeneity within and between tissues, generating genetic mosaicism in the body and the risk of cancer that could arise from those mutations (1). Randomness of DNA mutations and epigenetic modifications during cell division results in different outcomes in the host. Spontaneous mutations could be harmless, beneficial, or deleterious to human cells, whereas accidental mutations are often harmful. Dynamics of DNA mutation and epigenetic modification mechanisms make cellular transformation an inevitable event.

Harmless somatic mutations have been reported in healthy hematopoietic stem cells of women with a constant mutation rate of four mutations per year or three mutations per cell division. These mutations were found in regions that were not evolutionarily conserved (2). Another example of harmless somatic mutations includes somatic mutations in the hypoxanthine-guanine phosphoribosyltransferase (hprt) gene in T cells of normal children. This is a V(D)J recombinase–mediated recombination event that is found in 30% to 35% of children under 5 years of age (3). The frequency of these specific changes is dramatically decreased in older children.

Beneficial somatic mutations constantly occur in cells of the immune system to maintain their effector function. For instance, somatic hypermutation in the variable regions of immunoglobulin genes is a major component of the process of affinity maturation, allowing diversification of B-cell receptors in recognizing numerous antigens and distinguishing self-antigens from foreign antigens (4). Lactose tolerance is also the result of beneficial mutations that create evolutionary polymorphism in lactase-phlorizin hydrolase, the enzyme responsible for hydrolysis of milk lactose into glucose and galactose. Lactose tolerance is found in around 35% of adults living in the world, mostly people with European ancestry (5). This enzyme is expressed during infancy, but after the weaning period is over, lactase production usually declines. However, 35% of human population continues to express lactase throughout adult life. Another beneficial mutation was reported in the *CCR5-delta32* gene, which can block the entry of human immunodeficiency virus (HIV) into $CD4^+$ T cells and protect the mutant carrier from AIDS (6). Beneficial mutations in a gene may progress to a harmful mutation. For instance, a point mutation in just one copy of the hemoglobin gene can protect the host from malaria (7), whereas two copies of the mutated hemoglobin gene cause sickle cell anemia. T-cell differentiation is also regulated through beneficial epigenetic modifications. Analysis of Th0, Th1, and Th2 cells indicated that the IFNγ and Th2 cytokine loci were not modified in Th0. In fact, active or repressive histone modifications in the cytokine locus determine Th1/Th2 differentiation (8).

Deleterious somatic mutations or epigenetic alterations result in cellular malignancy and cancer. Changes in methylation patterns or histone deacetylation are hallmarks of epigenetic modulation, which can alter gene expression. As methylation and

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histone deacetylation rates are faster than genetic mutation rates, epigenetic alterations could occur very quickly during the lifetime of an individual. DNA hypermethylation can inactivate the genes that are responsible for DNA damage response and repair, facilitating the establishment of cancer. It was reported that each environmental insult alters a specific checkpoint that it triggers and increases risk of certain cancers (9). For instance, bisphenol A, a plasticizer used for manufacturing polycarbonate plastics, can leach from plastics into food and water, disrupts mitotic progression, and increases risk of prostate and breast cancers (10, 11). Environmental insults often result in deleterious mutations, although subsequent induction of tumor suppressor genes inhibits growth of malignant cells and prevents cancer development. For instance, p53 is activated in response to DNA damage, hypoxia, and nucleotide deprivation. Activation of p53 leads to cell-cycle arrest, apoptosis, or DNA repair to restore the integrity of cells. However, loss of p53 function through mutations could lead to survival of malignant cells. Once the process of malignancy is completed, transformed cells cannot necessarily form cancer. Fortunately, metastasis suppressor genes could still limit invasiveness of malignant cells, and mutant protein antigens expressed by malignant cells can be specifically recognized and attacked by the immune system, resulting in the maintenance of malignant dormancy. However, tumor cells that arise from normal cells have adapted similar evolutionary mechanisms of survival that enable them to escape immune surveillance. These mechanisms have been well explained by the tumor immunoediting theory (12). In fact, humans have evolved two major mechanisms of survival from tumor-inducing environmental insults. These include (i) tumor-intrinsic mechanisms regulated by metastasis suppressor genes and cell-cycle checkpoint molecules, which could inhibit proliferation of malignant cells and establish tumor dormancy; and (ii) tumor-extrinsic mechanisms regulated by the immune surveillance, which could either eliminate or inhibit nascent transformed cells. Inhibition of transformed cells could, in turn, facilitate the establishment of immunogenic tumor dormancy. In fact, Th1 cells have been reported to inhibit HER2-positive tumor growth such that loss of anti-HER2 or anti-HER3 Th1 response was found to be associated with tumor recurrence (13, 14).

Cellular Dormancy Is an Evolutionary Conserved Mechanism of Survival

In a thorough review of the evidence of cancer dormancy, Aguirre-Ghiso suggested that cellular dormancy is an evolutionary conserved mechanism among organisms to help them adapt to stress and survive a hostile environment (15). In *Caenorhabditis elegans*, pathways that sense stress will induce cellular dormancy or growth arrest and result in resistance of larvae to nutritional deprivation (16). Mycobacterium tuberculosis and HIV survive in human cells by entering into a dormant, latent state (17, 18). Mammalian adult stem cells are also in a state of quiescent dormancy until they receive specific signals, such as tissue injury, to exit from dormancy and proliferate (19, 20). It was also reported that in the absence of antigen, memory T cells enter a state of dormancy associated with low energy utilization and proliferation to survive until they receive stimulatory signals during a subsequent infection (21). In fact, cellular dormancy is the mechanism by which memory T cells survive nearly throughout the lifetime to protect an individual from recall infections. Memory T cells could escape from dormancy during recall infection and generate effector T cells with the ability to proliferate (21). Although the mechanisms of cellular dormancy are not fully understood, stress-induced autophagy could lead to cellular dormancy. In T cells, macroautophagy is upregulated just before the contraction phase, when T cells stop dividing and the pathogen has been cleared (22). Autophagy-deficient $CD8^+$ T cells were found to be defective in generating memory phenotypes that are usually in the state of dormancy (22). Given that malignant cells arise from normal cells, it is reasonable to suggest that tumor dormancy recapitulates evolutionarily conserved mechanism of adaptation,that is, cellular dormancy to survive hostile microenvironment. This property facilitates the establishment of treatment-induced tumor dormancy following conventional cancer therapies or immunotherapy (23–25). In fact, IFNγ produced by tumor-reactive T cells induces tumor cell apoptosis as well as tumor cell dormancy, and relapse associated with tumor immunoediting, simultaneously (26, 27). Such a paradoxical response by tumor cells to the immune response was shown to be due to the inherent heterogeneity of mammary tumor cells for the expression of IFNγ Rα (28).

Local or Metastatic Tumor Dormancy Is Present Prior to Cancer

Patients with early-stage cancer do not die from primary cancer, which tends to be responsive to therapy, but rather as a result of distant recurrence of the tumor in the form of advanced stage diseases. Twenty percent to 45% of patients with breast or prostate cancer end up with distant recurrence of the disease years or decades after successful treatment of their primary cancer (29, 30). This phenomenon can be explained by cancer dormancy, a stage in which residual disease is present but remains asymptomatic, and most often, undetectable. Tumor dormancy is present in almost all cancers, particularly breast cancer. Emerging evidence suggests that local and metastatic tumor dormancy precede primary cancer and distant tumor metastasis, respectively.

Local tumor dormancy prior to establishment of primary cancer

The concept that local malignant dormancy precedes primary cancer is supported by the existence of "cancer without disease" (31), tissue-specific control of malignant dormancy (32), as well as clinical evidence in support of the existence of natural tumor dormancy in healthy individuals highlighted in the recent review articles (32, 33). For instance, postmortem examination of random sections of autopsied prostate tissues from men who did not have cancer revealed frequent "small carcinomata" in 14% of prostate specimens (34, 35). More recent studies revealed the presence of *in situ* carcinoma in 9%, 27%, and 34% of cancer-free men in their 20s, 30s, and 40s, respectively (35). Postmortem examination of women in their 40s showed a similar frequency (39%) of histologic breast cancers (36), although only 1% of women in this age range get breast cancer. Interestingly, all autopsied individuals ages 50 to 70 had *in situ* carcinomas in the thyroid gland (37), whereas the incidence of thyroid cancer in this age group is only 0.1% (31). Frequency of dormant lung cancer was lower, accounting for 1% of autopsied specimens from individuals who were cancer free (38). Pancreatic intraepithelial neoplasia being in a dormant state is remarkably common, particularly in cancer-free elderly (39). They contain mutations in the same genes that are mutated in invasive pancreatic cancer

(40, 41), suggesting the state of malignant dormancy. These data suggest that local tumor dormancy precedes primary cancer development and that tumor cells could remain dormant for the lifetime of an individual without ever causing cancer. Very recently, circulating tumor DNA carrying P53 mutations has been reported in healthy individuals (42), again suggesting that malignancy is present prior to the development of primary cancer.

Metastatic tumor dormancy prior to establishment of primary cancer

For the past century, it has been assumed that tumor metastasis follows a stepwise process from primary tumor to the regional lymph nodes and then distant organs. This classical understanding of tumor metastasis has guided removal of the draining lymph nodes during conventional therapies. Recent evidence from patients with solid malignancies indicates that metastasis is a very early event such that even small tumors (<5 mm) can establish metastasis long before they become detectable at the primary site. This phenomenon is defined as early dissemination but late metastasis, because metastatic cells could lie dormant for even a decade and then reemerge as metastatic disease (43, 44). More recently, the observations made in two groups of cancer patients have further challenged the classical view of tumor metastasis. The first group of patients comprises those with metastatic lesions either before the primary tumor became clinically detectable, or when harboring primary cancer at a very early stage without local invasion. For instance, patients with stage M0 breast cancer could relapse after complete resection of their primary tumor, and their metastatic tumor had significantly fewer genetic abnormalities than the primary tumor (45). Studies in melanoma model demonstrated that tumor cells were disseminated throughout the body even before primary tumor became clinically detectable (46). Mechanistic studies revealed that in early lesions prior to establishment of breast cancer, there was a subpopulation of early cancer cells that spread to distant organs. Further studies demonstrated that progesterone-induced signaling induces dissemination of malignant cells from early lesions shortly after HER2 activation and prior to breast cancer development (43). Another group of cancer patients comprises those with cancer of unknown primary. Up to 5% of all cancer diagnoses are classified as cancer of unknown primary (47). In these patients, primary cancer could not be identified after histopathologic review of biopsy material and CT scan, but full-body imaging identified metastatic lesions that were confirmed by biopsy. Even a postmortem examination of a small group of patients with cancer of unknown primary revealed only 55% to 85% of the primaries, which were very small asymptomatic tumors in the lung, gut, and kidney. The remaining were autopsy-negative primary sites with detectable metastatic lesions (48). Metastatic cancers of unknown primary were reported in cervical carcinoma, renal cancer, breast cancer, colorectal cancer, lung cancer, liver cancer, pancreatic cancer, and ovarian cancer. Cancer of unknown primary is a clinical puzzle for oncologists and could be explained by the notion that circulating tumor cells must be present very early during the process of malignancy and reside in distant organs in a dormant state prior to the establishment of primary tumor. These dormant cells can then establish metastatic cancer prior to the detection of primary cancer (cancer of unknown primary) or relapse at distant organs after successful treatment of the primary cancer. Perhaps, metastasis suppressor genes are involved in maintaining tumor dormancy at distant sites. Although both metastasis suppressor genes and tumor suppressor genes are tumor cell–intrinsic mechanisms of survival, the former is distinct from the latter in that metastasis suppressor genes maintain metastatic cells in a dormant state without affecting the growth of the primary tumor (49–52). On the other hand, tumor suppressor genes undergo mutation or epigenetic alterations during tumorigenesis or latency. Each cancer type appears to have distinct metastasis suppressor genes. For instance, *Nm23* and *BRMS1* are involved in breast cancer, *KAI1*, *MKK4*, *Rkip*, *RHOGDI2*, and *Drg-1* are involved in prostate cancer, and *TXNIP*, *CRSP3*, and *KISS1* are involved in melanoma (50). Failure of tumor cell–intrinsic mechanisms of survival, including metastasis suppressor genes, tumor suppressor genes, and cell-cycle checkpoint molecules, does not immediately result in cancer because cell-extrinsic mechanisms mediated by the immunosurveillance could still support tumor dormancy by inhibiting the growth of nascent transformed cells (12). This mechanism has been demonstrated by the equilibrium phase of tumor immunoediting (53). However, escape from immune-mediated tumor dormancy could lead to distant recurrence of cancer (33).

Chronic Inflammation Awakens Dormant Malignant Cells and Results in Cancer

A substantial body of evidence supports the role of chronic inflammation in cancer development. For instance, colon carcinoma is associated with inflammatory bowel disease, esophageal cancer is associated with acid reflux esophagitis, liver cancer is associated with fatty liver disease and hepatitis, bladder cancer is associated with cystitis and schistosomiasis, and stomach cancer is associated with chronic *Helicobacter* infection. It has long been thought that chronic inflammation facilitates cell transformation and malignancy by increasing free radicals. During inflammation, there are high levels of reactive oxygen and nitrogen species (RONS), which can induce mutagenic DNA lesions. RONS also induce DNA double-strand breaks, which can also be potently mutagenic if not accurately repaired. However, detection of malignant cells in postmortem autopsy specimens of individuals in the absence of any chronic inflammation outcasts a cause–effect relationship between chronic inflammation and cancer (31, 33, 39, 42). In addition, not all individuals with chronic inflammatory diseases end up with cancer. Tumorigenic manifestation of chronic inflammation could be due to its role in awakening dormant malignant cells rather than causing malignancy. To this end, the incidence and the type of cancer in individuals could be determined by the presence of malignant dormancy that each organ might carry to communicate with chronic inflammatory environment. In fact, chronic inflammation supports angiogenesis, which is an important factor in the promotion of growth of dormant micrometastasis (54). For instance, there is a strong correlation between inflammation and recurrence of endometrial cancer (55), oral cancer (56), breast cancer (57, 58), and tumor escape from dormancy induced by the inflammatory cytokine IFNγ (27, 28, 59, 60). In addition, data from patients with tumor recurrence after successful treatment of their primary cancer support this hypothesis. For instance, in a multisite study of 734 breast cancer survivors, high levels of circulating acute phase proteins (APP) were associated with distant recurrence of cancer (61). Therefore, posttreatment monitoring of serum inflammatory markers, such as APP, C-reactive

protein, and IL6, could be of prognostic value for predicting risk of breast cancer recurrence.

Escape from Cell-Intrinsic and Cell-Extrinsic Mechanisms of Tumor Dormancy Results in Distant Recurrence of Cancer

Like normal cells, malignant cells that lie dormant could evolve and escape from dormancy. Such evolutionary mechanisms could be facilitated by chronic inflammation that induces mutations and epigenetic alterations in metastasis suppressor genes. This, in turn, abolishes tumor cell–intrinsic mechanisms of metastatic dormancy, resulting in distant recurrence of the disease in the form of advanced stage cancer. Fortunately, mutant protein antigens expressed by malignant cells can be specifically recognized and attacked by the immune system, thereby providing tumor cell–extrinsic mechanisms for the maintenance of metastatic dormancy. In fact, immunogenic tumor dormancy has been suggested to be a key mechanism of tumor dormancy (33, 62). For instance, tumor cells that were disseminated prior to the formation of primary cancer were in the state of dormancy in the lung as a result of the cytostatic function of $CD8^+$ T cells (46). Depletion of $CD8^+$ T cells resulted in the outgrowth and relapse of metastatic dormant cells (46). Studies in an animal model of pancreatic cancer demonstrated that circulating pancreatic cells underwent epithelial-to-mesenchymal transition (EMT) and seeded the liver. EMT and invasiveness were most abundant at inflammatory sites such that treatment with the immunosuppressive drug, dexamethasone, abrogated tumor invasiveness. The authors suggested that inflammation enhances cancer progression in part by facilitating EMT (63). It was also reported that localized inflammation in the lungs triggers escape from dormancy, which develop into macroscopic metastases (64). However, dormant tumor cells that arise from normal cells possess similar evolutionary mechanisms of survival that could result in escaping from immunosurveillance. Thus far, two types of tumor dormancy have been reported; these include $Ki67^-$ quiescent dormancy and $Ki67^{low}$ indolent dormancy (27). The latter is maintained through a balance between sluggish cell proliferation and cell death. Interestingly, an indolent, but not a quiescent, type of tumor dormancy was found to be able to evolve through immunoediting and escape from the immune response. The inflammatory cytokine, IFNγ, was a key factor in facilitating tumor immunoediting (27). In fact, IFNγ-producing Th1 cells can induce apoptosis and HER2 loss in murine and human breast cancer (60). Immune escape mechanisms include, but are not limited to, tumor antigen loss, expression of PD-L1, loss or downregulation of MHC class I, and induction of MDSCs and/or Tregs. Therefore, distant recurrence of cancer in some but not all cancer survivors could depend on the state of dormancy, that is, quiescent or indolent.

In summary, (i) cellular transformation is unavoidable in biological systems; (ii) malignant cells often enter the state of dormancy to survive environmental insults; (iii) malignant dormant cells are best targets for the prevention of metastasis, as suggested in a recent review of by Ghajar (65); and (iv) malignant dormant cells could evolve, escape from the immune surveillance or other cancer therapies, and relapse. Therefore, attempts to destroy and eliminate cancer without any risk of relapse would be unfruitful. Rather, we need to develop new therapeutic strategies to control malignant cells through retaining them in the state of residual dormancy and preventing distant recurrence of the disease. This could be achieved by immunotherapeutic targeting of dormant cells, because all other currently available cancer therapies are toxic with off-target effects, whereas immune cells could establish memory against dormant tumor antigens such as mutated tumor antigens, and keep them dormant for the lifetime of an individual.

Disclosure of Potential Conflicts of Interest

M.H. Manjili is a consultant/advisory board member for Getting To Know Cancer.

Disclaimer

Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Department of Defense.

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References

1. Frank SA. Evolution in health and medicine Sackler colloquium: somatic evolutionary genomics: mutations during development cause highly variable genetic mosaicism with risk of cancer and neurodegeneration. Proc Natl Acad Sci U S A 2010;107Suppl 1:1725–30.
2. Holstege H, Pfeiffer W, Sie D, Hulsman M, Nicholas TJ, Lee CC, et al. Somatic mutations found in the healthy blood compartment of a 115-yr-old woman demonstrate oligoclonal hematopoiesis. Genome Res 2014; 24:733–42.
3. Finette BA, Poseno T, Albertini RJ. V(D)J recombinase-mediated HPRT mutations in peripheral blood lymphocytes of normal children. Cancer Res 1996;56:1405–12.
4. Reed JH, Jackson J, Christ D, Goodnow CC. Clonal redemption of autoantibodies by somatic hypermutation away from self-reactivity during human immunization. J Exp Med 2016;213:1255–65.
5. Lewinsky RH, Jensen TG, Moller J, Stensballe A, Olsen J, Troelsen JT. T-13910 DNA variant associated with lactase persistence interacts with Oct-1 and stimulates lactase promoter activity *in vitro*. Hum Mol Genet 2005; 14:3945–53.
6. Stephens JC, Reich DE, Goldstein DB, Shin HD, Smith MW, Carrington M, et al. Dating the origin of the CCR5-Delta32 AIDS-resistance allele by the coalescence of haplotypes. Am J Hum Genet 1998;62:1507–15.
7. Goncalves BP, Gupta S, Penman BS. Sickle haemoglobin, haemoglobin C and malaria mortality feedbacks. Malar J 2016;15:26.
8. Fields PE, Kim ST, Flavell RA. Cutting edge: changes in histone acetylation at the IL-4 and IFN-gamma loci accompany Th1/Th2 differentiation. J Immunol 2002;169:647–50.
9. Breivik J. The evolutionary origin of genetic instability in cancer development. Semin Cancer Biol 2005;15:51–60.

10. Ho SM, Tang WY, Belmonte de Frausto J, Prins GS. Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. Cancer Res 2006;66:5624–32.
11. Morgan M, Deoraj A, Felty Q, Roy D. Environmental estrogen-like endocrine disrupting chemicals and breast cancer. Mol Cell Endocrinol 2016 Oct 4. [Epub ahead of print].
12. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. Annu Rev Immunol 2004;22:329–60.
13. Datta J, Fracol M, McMillan MT, Berk E, Xu S, Goodman N, et al. Association of depressed anti-HER2 T-helper type 1 response with recurrence in patients with completely treated HER2-positive breast cancer: role for immune monitoring. JAMA Oncol 2016;2:242–6.
14. Fracol M, Datta J, Lowenfeld L, Xu S, Zhang PJ, Fisher CS, et al. Loss of anti-HER-3 CD4+ T-helper type 1 immunity occurs in breast tumorigenesis and is negatively associated with outcomes. Ann Surg Oncol 2017;24:407–17.
15. Aguirre-Ghiso JA. Models, mechanisms and clinical evidence for cancer dormancy. Nat Rev Cancer 2007;7:834–46.
16. Fukuyama M, Rougvie AE, Rothman JH. *C. elegans* DAF-18/PTEN mediates nutrient-dependent arrest of cell cycle and growth in the germline. Curr Biol 2006;16:773–9.
17. Lipworth S, Hammond RJ, Baron VO, Hu Y, Coates A, Gillespie SH. Defining dormancy in mycobacterial disease. Tuberculosis 2016;99: 131–42.
18. Matsuda Y, Kobayashi-Ishihara M, Fujikawa D, Ishida T, Watanabe T, Yamagishi M. Epigenetic heterogeneity in HIV-1 latency establishment. Sci Rep 2015;5:7701.
19. Sosa MS, Bragado P, Aguirre-Ghiso JA. Mechanisms of disseminated cancer cell dormancy: an awakening field. Nat Rev Cancer 2014;14:611–22.
20. Scognamiglio R, Cabezas-Wallscheid N, Thier MC, Altamura S, Reyes A, Prendergast AM, et al. Myc depletion induces a pluripotent dormant state mimicking diapause. Cell 2016;164:668–80.
21. Dalai SK, Khoruzhenko S, Drake CG, Jie CC, Sadegh-Nasseri S. Resolution of infection promotes a state of dormancy and long survival of CD4 memory T cells. Immunol Cell Biol 2011;89:870–81.
22. Xu X, Araki K, Li S, Han JH, Ye L, Tan WG, et al. Autophagy is essential for effector CD8(+) T cell survival and memory formation. Nat Immunol 2014;15:1152–61.
23. Jinno-Oue A, Shimizu N, Hamada N, Wada S, Tanaka A, Shinagawa M, et al. Irradiation with carbon ion beams induces apoptosis, autophagy, and cellular senescence in a human glioma-derived cell line. Int J Radiat Oncol Biol Phys 2010;76:229–41.
24. Romero I, Garrido C, Algarra I, Collado A, Garrido F, Garcia-Lora AM. T lymphocytes restrain spontaneous metastases in permanent dormancy. Cancer Res 2014;74:1958–68.
25. Dai Y, Wang L, Tang J, Cao P, Luo Z, Sun J, et al. Activation of anaphase-promoting complex by p53 induces a state of dormancy in cancer cells against chemotherapeutic stress. Oncotarget 2016;7:25478–92.
26. Farrar JD, Katz KH, Windsor J, Thrush G, Scheuermann RH, Uhr JW, et al. Cancer dormancy. VII. A regulatory role for CD8+ T cells and IFN-gamma in establishing and maintaining the tumor-dormant state. J Immunol 1999;162:2842–9.
27. Payne KK, Keim RC, Graham L, Idowu MO, Wan W, Wang XY, et al. Tumor-reactive immune cells protect against metastatic tumor and induce immunoediting of indolent but not quiescent tumor cells. J Leukoc Biol 2016; 100:625–35.
28. Kmieciak M, Payne KK, Wang XY, Manjili MH. IFN-gamma ralpha is a key determinant of CD8+ T cell-mediated tumor elimination or tumor escape and relapse in FVB mouse. PLoS One 2013;8:e82544.
29. Karrison TG, Ferguson DJ, Meier P. Dormancy of mammary carcinoma after mastectomy. J Natl Cancer Inst 1999;91:80–5.
30. Weckermann D, Muller P, Wawroschek F, Harzmann R, Riethmuller G, Schlimok G. Disseminated cytokeratin positive tumor cells in the bone marrow of patients with prostate cancer: detection and prognostic value. J Urol 2001;166:699–703.
31. Folkman J, Kalluri R. Cancer without disease. Nature 2004;427:787.
32. Bissell MJ, Hines WC. Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression. Nat Med 2011;17: 320–9.
33. Manjili MH. The inherent premise of immunotherapy for cancer dormancy. Cancer Res 2014;74:6745–9.
34. Rich AR. Classics in oncology. On the frequency of occurrence of occult carcinoma of the prostate: Arnold rice rich, M.D., journal of urology 33:3, 1935. CA Cancer J Clin 1979;29:115–9.
35. Sakr WA, Haas GP, Cassin BF, Pontes JE, Crissman JD. The frequency of carcinoma and intraepithelial neoplasia of the prostate in young male patients. J Urol 1993;150:379–85.
36. Nielsen M, Thomsen JL, Primdahl S, Dyreborg U, Andersen JA. Breast cancer and atypia among young and middle-aged women: a study of 110 medicolegal autopsies. Br J Cancer 1987;56:814–9.
37. Harach HR, Franssila KO, Wasenius VM. Occult papillary carcinoma of the thyroid. A "normal" finding in Finland. A systematic autopsy study. Cancer 1985;56:531–8.
38. Manser RL, Dodd M, Byrnes G, Irving LB, Campbell DA. Incidental lung cancers identified at coronial autopsy: implications for overdiagnosis of lung cancer by screening. Respir Med 2005;99:501–7.
39. Hruban RH, Maitra A, Goggins M. Update on pancreatic intraepithelial neoplasia. Int J Clin Exp Pathol 2008;1:306–16.
40. Goggins M, Hruban RH, Kern SE. BRCA2 is inactivated late in the development of pancreatic intraepithelial neoplasia: evidence and implications. Am J Pathol 2000;156:1767–71.
41. Luttges J, Schlehe B, Menke MA, Vogel I, Henne-Bruns D, Kloppel G. The K-ras mutation pattern in pancreatic ductal adenocarcinoma usually is identical to that in associated normal, hyperplastic, and metaplastic ductal epithelium. Cancer 1999;85:1703–10.
42. Fernandez-Cuesta L, Perdomo S, Avogbe PH, Leblay N, Delhomme TM, Gaborieau V, et al. Identification of circulating tumor DNA for the early detection of small-cell lung cancer. EBioMedicine 2016;10:117–23.
43. Hosseini H, Obradovic MM, Hoffmann M, Harper KL, Sosa MS, Werner-Klein M, et al. Early dissemination seeds metastasis in breast cancer. Nature 2016 Dec 14. [Epub ahead of print].
44. Harper KL, Sosa MS, Entenberg D, Hosseini H, Cheung JF, Nobre R, et al. Mechanism of early dissemination and metastasis in Her2+ mammary cancer. Nature 2016 Dec 14. [Epub ahead of print].
45. Schmidt-Kittler O, Ragg T, Daskalakis A, Granzow M, Ahr A, Blankenstein TJ, et al. From latent disseminated cells to overt metastasis: genetic analysis of systemic breast cancer progression. Proc Natl Acad Sci U S A 2003;100: 7737–42.
46. Eyles J, Puaux AL, Wang X, Toh B, Prakash C, Hong M, et al. Tumor cells disseminate early, but immunosurveillance limits metastatic outgrowth, in a mouse model of melanoma. J Clin Invest 2010;120:2030–9.
47. Pavlidis N, Khaled H, Gaafar R. A mini review on cancer of unknown primary site: a clinical puzzle for the oncologists. J Adv Res 2015;6:375–82.
48. Mayordomo JI, Guerra JM, Guijarro C, Garcia-Prats MD, Gomez A, Lopez-Brea M, et al. Neoplasms of unknown primary site: a clinicopathological study of autopsied patients. Tumori 1993;79:321–4.
49. Horak CE, Lee JH, Marshall JC, Shreeve SM, Steeg PS. The role of metastasis suppressor genes in metastatic dormancy. APMIS 2008;116:586–601.
50. Hedley BD, Allan AL, Chambers AF. Tumor dormancy and the role of metastasis suppressor genes in regulating ectopic growth. Future Oncol 2006;2:627–41.
51. Nash KT, Phadke PA, Navenot JM, Hurst DR, Accavitti-Loper MA, Sztul E, et al. Requirement of KISS1 secretion for multiple organ metastasis suppression and maintenance of tumor dormancy. J Natl Cancer Inst 2007;99:309–21.
52. Seraj MJ, Samant RS, Verderame MF, Welch DR. Functional evidence for a novel human breast carcinoma metastasis suppressor, BRMS1, encoded at chromosome 11q13. Cancer Res 2000;60:2764–9.
53. Teng MW, Swann JB, Koebel CM, Schreiber RD, Smyth MJ. Immune-mediated dormancy: an equilibrium with cancer. J Leukoc Biol 2008;84: 988–93.
54. Folkman J. Role of angiogenesis in tumor growth and metastasis. Semin Oncol 2002;29:15–8.
55. Machida H, De Zoysa MY, Takiuchi T, Hom MS, Tierney KE, Matsuo K. Significance of monocyte counts at recurrence on survival outcome of women with endometrial cancer. Int J Gynecol Cancer 2017;27:302–10.
56. Okubo M, Kioi M, Nakashima H, Sugiura K, Mitsudo K, Aoki I, et al. M2-polarized macrophages contribute to neovasculogenesis, leading to relapse of oral cancer following radiation. Sci Rep 2016;6:27548.
57. Hughes R, Qian BZ, Rowan C, Muthana M, Keklikoglou I, Olson OC, et al. Perivascular M2 macrophages stimulate tumor relapse after chemotherapy. Cancer Res 2015;75:3479–91.

58. Bowers LW, Maximo IX, Brenner AJ, Beeram M, Hursting SD, Price RS, et al. NSAID use reduces breast cancer recurrence in overweight and obese women: role of prostaglandin-aromatase interactions. Cancer Res 2014;74:4446–57.
59. Hallermalm K, Seki K, De Geer A, Motyka B, Bleackley RC, Jager MJ, et al. Modulation of the tumor cell phenotype by IFN-gamma results in resistance of uveal melanoma cells to granule-mediated lysis by cytotoxic lymphocytes. J Immunol 2008; 180:3766–74.
60. Namjoshi P, Showalter L, Czerniecki BJ, Koski GK. T-helper 1-type cytokines induce apoptosis and loss of HER-family oncodriver expression in murine and human breast cancer cells. Oncotarget 2016 Jun 25. [Epub ahead of print].
61. Cole SW. Chronic inflammation and breast cancer recurrence. J Clin Oncol 2009;27:3418–9.
62. Baxevanis CN, Perez SA. Cancer dormancy: a regulatory role for endogenous immunity in establishing and maintaining the tumor dormant state. Vaccines 2015;3:597–619.
63. Rhim AD, Mirek ET, Aiello NM, Maitra A, Bailey JM, McAllister F, et al. EMT and dissemination precede pancreatic tumor formation. Cell 2012;148:349–61.
64. De Cock JM, Shibue T, Dongre A, Keckesova Z, Reinhardt F, Weinberg RA. Inflammation triggers Zeb1-dependent escape from tumor latency. Cancer Res 2016;76:6778–84.
65. Ghajar CM. Metastasis prevention by targeting the dormant niche. Nat Rev Cancer 2015;15:238–47.

Tumor Dormancy and Relapse: From a Natural Byproduct of Evolution to a Disease State

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Role of Tregs in Cancer Dormancy or Recurrence

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ABSTRACT

The immunogenic tumor dormancy has been demonstrated in animal models of cancer, which can explain clinical observations such as an increased incidence of cancer following organ transplantation. The role of immune cell populations in the maintenance of, or escape from, tumor dormancy and subsequent recurrence is poorly understood. Here, we provide a review of literature related to the contribution of Tregs in tumor dormancy or recurrence. Based on clinical results, we suggest that anecdotal reports on the association of human Tregs with poor prognosis are circumstantial rather than implying a cause–effect direction. This could be due to a disparity among patients in harboring multiple factors associated with tumor immunoediting and immune evasion mechanisms.

KEYWORDS

Regulatory T cells; tumor dormancy; tumor immunoediting

Introduction

Patients with cancer are always at risk of developing distant recurrence of the disease years or decades after successful treatment of their primary cancer. This phenomenon can be explained by cancer dormancy, a stage that residual disease is present but remains asymptomatic, and most often, undetectable. Mechanisms that establish and maintain tumor dormancy or result in tumor recurrence by escaping from dormancy are poorly understood. Cancer dormancy can be explained by two distinct but interrelated mechanisms. These include (i) immunogenic tumor dormancy controlled by the immune system, and (ii) non-immunogenic or cellular tumor dormancy controlled by a balanced proliferation and death or by cellular quiescence (Manjili 2014; Manjili and Payne 2015). There is also another mechanism of tumor dormancy named angiogenic dormancy (Ghajar et al. 2013), which can be explained in the context of immunogenic or non-immunogenic dormancy. Tumor dormancy is present in almost all cancers, particularly breast cancer. Up to 30% of early-stage breast cancers with no evidence of metastasis will relapse in distant organs less than a decade after the treatment of primary cancer (Aguirre-Ghiso 2007). Therefore, adjuvant chemotherapy that kills cycling tumor cells is an option after successful resection of the tumor or lumpectomy. Yet, chemotherapy has been met with limited success as it reduces metastatic recurrence by only 30% at 10 years (Demicheli et al. 2005). While many tumor clones undergo apoptosis upon chemotherapy, some other

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tumor clones escape from apoptosis, become quiescent, and lie dormant (Aguirre-Ghiso 2007; Demicheli et al. 2005). Dormant breast cancer cells have been detected as disseminated tumor cells (DTC) that reside in distant organs, as well as circulating tumor cells (CTC) that can be detected in the bloodstream. In our recent review, we have discussed clinical and preclinical evidence that support the existence of tumor dormancy in healthy individuals, that is, natural dormancy as well as in cancer survivors, that is, treatment-induced dormancy (Manjili 2014; Manjili and Payne 2015). Presence of CTC or DTC in healthy individuals suggests that dormant tumor cells are not always derived from the primary tumors. It is yet to be determined whether CTCs in cancer survivors are derived from their primary tumors in response to conventional therapies, or they are secondary dormant cells that did not respond to conventional therapies. However, preclinical studies suggest that dormant tumor cells that establish distant metastasis are derived from the primary tumor (Aceto et al. 2014). Here, we review clinical and experimental evidence to understand the role of Tregs in tumor dormancy or recurrence.

T cells and immunogenic tumor dormancy

Immunogenic tumor dormancy and recurrence can be explained in the context of tumor immunoediting proposed by Dr. Schreiber (Dunn et al. 2002). According to the tumor immunoediting theory, immunogenic tumor clones can either be eliminated, elimination phase; or lie dormant and remain in check, equilibrium phase, or tumor dormancy, by tumor-specific immune responses. Dormant tumor cells could eventually relapse because of tumor escape such as antigen loss or immune evasion. This process is called three *E*s (elimination, equilibrium, and escape) of tumor immunoediting (Dunn, Old, and Schreiber 2004). Immunogenic tumor dormancy is also supported by spontaneous regression of highly immunogenic cancers associated with infiltrating CD4+ T cells (Halliday et al. 1995), and development of melanoma or cancers with a viral etiology in organ transplant recipients. In patients with basal cell carcinoma who participated in clinical trial of IFN-α, 20% of patients on the placebo arm of the trial experienced spontaneous regression of the tumor (Printz 2001), suggesting that about 20% of basal cell carcinomas undergo complete spontaneous regression. However, they did not investigate infiltrating T cell subsets to determine whether the ratio of effector T cells to Tregs was greater in 20% of patients with spontaneous tumor regression compared with those who failed to reject their tumor. The first report on spontaneous regression of breast cancer associated with extensive infiltration of T cells was published in 2014 (Tokunaga et al. 2014), again infiltrating T cell subsets were not characterized. Very recently, Dickerson et al. reported three cases that had spontaneous regression of renal cell carcinoma without any therapeutic interventions (Dickerson, Davenport, and Liu 2015). A patient with AIDS who had non-small cell lung cancer (NSCLC) experienced spontaneous regression of the tumor after immune reconstitution (Menon and Eaton 2015), suggesting the involvement of the immune system in tumor regression. These observations suggest that T cells can induce regression of immunogenic tumors.

IFN-γ producing CD8+ T cells have been shown to have a dual function, inducing tumor cell apoptosis – elimination – and inhibiting tumor cell growth – equilibrium (Kmieciak et al. 2011, 2013; Farrar et al. 1999). The latter can establish tumor dormancy, which could lead to tumor antigen loss and recurrence (Kmieciak et al. 2007; Payne et al. 2016). IFN-γ appears to be a key cytokine for the establishment of tumor dormancy, as well as the induction of epigenetic changes in tumor cells, leading to tumor antigen loss

and upregulation of PD-L1 in tumor cells (Payne et al. 2016; Kmieciak et al. 2013). We have recently reported two types of tumor dormancy, which include an indolent dormancy characterized by a balanced cell proliferation and death, and a quiescent dormancy characterized by lack of cell proliferation (Payne et al. 2016). We also showed that an indolent, but not a quiescent, tumor dormancy could eventually escape from immunogenic dormancy, and relapse (Payne et al. 2016). Escape from immunologic tumor dormancy and subsequent cancer development could also occur following immune suppression. For instance, organ recipients from healthy donors developed tumor in the organ following immunosuppression (Ali and Lear 2012). The US Scientific Registry of Transplant Recipients, which included 175,732 patients with solid organ transplantation (1987–2008) revealed 381 cases of melanoma in the recipients and an increased risk of 2.6 times higher than that of the general population (Engels et al. 2011). Similar results were obtained from a large combined Australasian registry-based prospective cohort study, which included 28,855 patients with up to 42 years of follow-up (Vajdic et al. 2006). These tumors generally had a viral etiology such as liver and cervical cancers, or were immunogenic tumors such as melanoma (Buell, Gross, and Woodle 2005; Penn 1988). These data suggest that highly immunogenic tumors that are in the state of immunogenic dormancy in the donor organ can establish cancer in the recipients because of the immune suppression to accept the graft. A meta-analysis of five population-based studies showed that the incidence of weakly immunogenic cancers, including breast, prostate, ovarian, and testicular cancers did not increase in transplant recipients (Vajdic and van Leeuwen 2009).

Tregs and tumor recurrence

Recent data have suggested that Tregs may be involved in the escape from immunogenic dormancy, and consequent recurrence. However, data related to the association of Tregs with poor outcome are controversial. Immunohistochemical analysis of FOXP3+Tregs in tumor specimens of 72 patients with early stage (I–III) breast cancer showed a significant correlation with a poor overall survival. Upon comparing paraffin-embedded tumors of multiple subsets, it was found that more aggressive subsets (lymph node metastases, immunopositivity for p53 and Ki-67) had higher numbers of FOXP3+ Tregs and lower numbers of CD8+ T cells. Further analysis indicated that an increase in FOXP3+Treg/CD4+ T-cell ratio was positively correlated with lymph node metastasis (Kim et al. 2013). Another group examined tumor specimens of 39 patients with glioblastoma (GBM) and demonstrated that a high ratio of CD8+ or CD3+ cells to FOXP3+ cells in primary tumor was associated with improved survival. There was no correlation between survival and a higher CD4 to FOXP3+ ratio (Sayour et al. 2015). These groups did not perform multicolor staining of tumor-infiltrating lymphocytes (TIL) to determine whether FOXP3+ cells were positive for CD4 and CD25. To this end, Suzuki et al. (2013) analyzed tumor specimens of 88 patients with colorectal cancer, and demonstrated that relatively low number of FOXP3+VEGFR2+ cells was significantly correlated with improved disease-free survival and overall survival. However, number of intratumoral FOXP3+ cells or FOXP3+VEGFR2– cells did not show significant correlation with disease-free survival and overall survival (Suzuki et al. 2013). FOXP3 is critical for the development and function of murine CD4+CD25+ Tregs (Haiqi, Yong, and Yi 2011). However, FOXP3 is also expressed in activated T cells upon stimulation of human CD4+CD25– T cells without conferring a

regulatory function (Kmieciak et al. 2009; Wang et al. 2007). Therefore, functional analyses as well as additional markers are needed to identify human Tregs. Although FOXP3+ T cells are hyporesponsive, they do not necessarily exhibit suppressor function (Ziegler 2007). Very recently, expression of the transcription factor Helios was proposed as a functional marker for naturally occurring Tregs. Muto et al. (2015) investigated the clinical significance of Helios expression in Tregs of 64 patients with NSCLC. They showed that patients with low levels of Helios expression in Tregs among their TILs had significantly poorer survival. Due to the variations in the markers used for the detection of human Tregs as well as different cancer types and contribution of other immune cells, it is difficult to determine the definitive role of human Tregs in tumor dormancy or recurrence.

In preclinical studies, Goding et al. (2013) reported that CD4+Foxp3+TRP-1 Tregs and chronically exhausted tumor-specific CD4+ T cells were increased during recurrence of B16 melanoma in mice. In order to determine if TRP-1 Foxp3+ tumor-specific CD4+ T cells cause tumor recurrence, TRP-1 transgenic mice were crossed with FoxP3-DTR (diphteria toxin (DT) reporter) transgenic mice allowing for cell-specific ablation of Foxp3+ Tregs and cell-specific tracking. Tregs were depleted with DT either during recurrence or immediately following successful primary treatment of B16 melanoma. Selective depletion was confirmed by flow cytometric analysis showing that effector T cells remained present during depletion and that GFP expression faithfully marked Foxp3-DTR TRP-1 Tregs. Depletion of Tregs alone failed to prevent recurrence, however, when PD-L1 was blocked in combination with Treg depletion, there was a significant regression in recurrence of B16 melanoma. Neither depletion of Tregs or blocking PD-L1 alone decreased recurrence, suggesting that neither is solely involved in mediating recurrence. It was also found that the number of CD8+ T cells remained unchanged in primary and recurrent tumors. In addition, high levels of IFN-γ and TNF-α were seen in non-relapsing mice and low levels in relapsing mice. In a mouse model of melanoma, it was also shown that Tregs suppress anti-tumor function of effector T cells (Jensen et al. 2012).

Tregs are dispensable during tumor dormancy or recurrence

The main function of Tregs has been shown to be maintaining immunological tolerance and protecting the host from excessive immune responses (Sakaguchi et al. 2008). In the gut, they produce IL-10 and TGF-β. IL-10 maintains intestinal homeostasis (Roers et al. 2004). TGF-β not only repairs mucosal injury but also preserves the integrity of the intestinal mucosa (Dignass and Podolsky 1993; Planchon et al. 1994) thereby protecting intestinal mucosa from inflammatory Th1 cells rather than just suppressing Th1 cells (Howe et al. 2005). TGF-β signaling is also critical for mucosal IgA production to protect the gut from pathogens (Borsutzky et al. 2004). Therefore, IL-10 and TGF-β producing Tregs play an active role in protecting the gut from injury, rather than acting passively by suppressing effector cells. In other words, they tend to exhibit a regulatory function rather than a suppressive function. Contribution of Tregs in tumor microenvironment can also be attributed to their regulatory function. For instance induced (i) Tregs enhance anti-tumor function of NK cells by increasing Fas ligand and perforin production while reducing IL-2 production in the absence of target cells (Bergmann et al. 2011). Although they appear to counteract cytotoxic function of T cells at the tumor site, they

do not tend to suppress cytostatic function of T cells, as anti-tumor CD8+ T cells can establish and maintain tumor dormancy even in the presence of Tregs (Gerber et al. 2013). Despite high levels of CD25 expression on Tregs, systemic administration of IL-2 has been shown to enhance anti-tumor responses (Whiteside et al. 1993; Atkins et al. 1999). In a mouse model of melanoma, the expression of IL-2 in the tumor microenvironment inhibits tumor growth despite enhancing Tregs and anti-inflammatory cytokines such as IL-10 (Gerber et al. 2013). These data suggest that Tregs are dispensable in suppressing anti-tumor immune responses that lead to escape from immunogenic dormancy and results in tumor relapse. An occasional correlation of Tregs with poor prognosis could

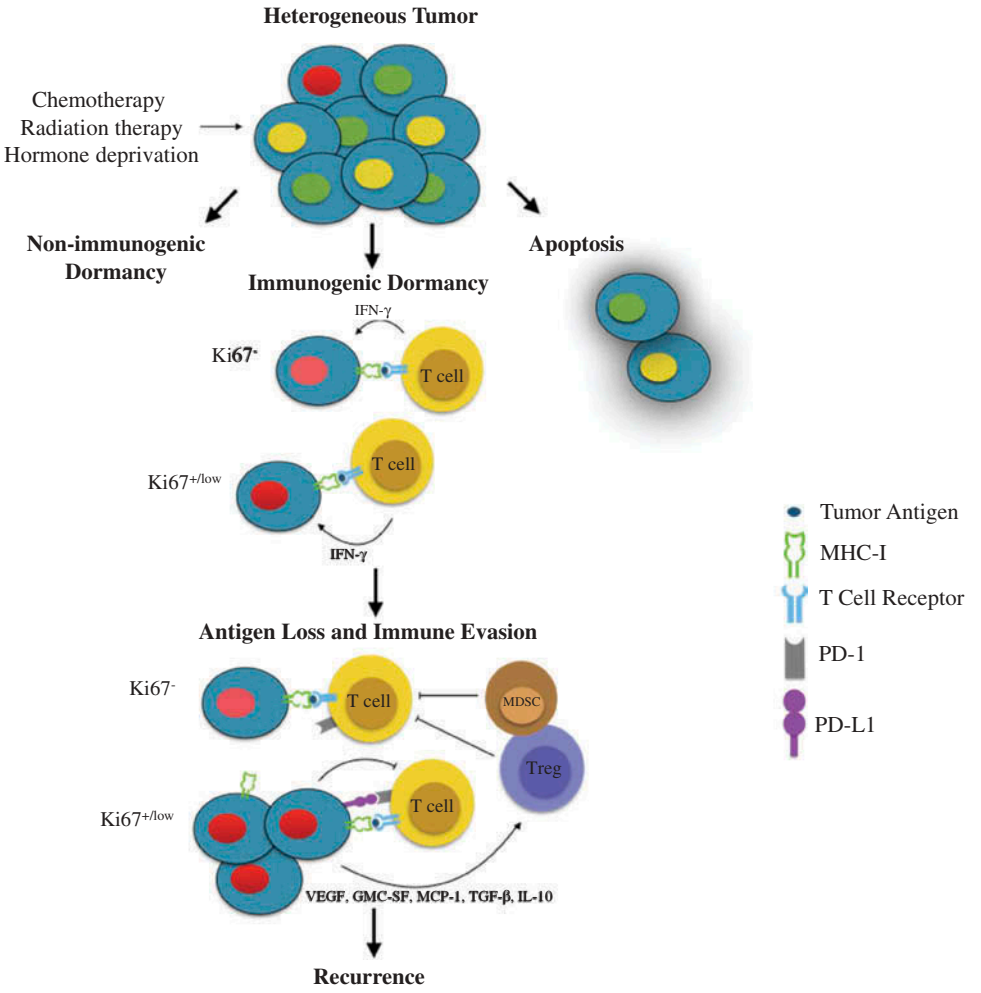

Figure 1. Tumor escape and immune evasion during immunogenic tumor dormancy. Dormant tumor cells could escape from dormancy and establish recurrence by undergoing immunoediting. Unlike quiescent dormant cells (Ki-67^{-}), indolent dormant cells (Ki-67$^{+/low}$) can be changed by IFN-γ producing T cells, and lose their tumor antigen and/or upregulate PD-L1 (tumor escape). Indolent dormant cells could also produce cytokines/chemokines that support Tregs and/or MDSCs, thereby suppressing anti-tumor immune responses (immune evasions). These events promote tumor recurrence.

be circumstantial due to the contribution of other factors such as MDSCs and immune checkpoint pathways.

In conclusion, escape from immunogenic tumor dormancy and subsequent relapse is due to tumor escape and immune evasion (Figure 1). Tumor escape is characterized by epigenetic changes in the indolent dormant cells mediated by IFN-γ producing T cells that induce tumor antigen loss (Kmieciak et al. 2007, 2011, 2013; Beatty and Paterson 2000). Immune evasion is characterized by: (i) increases of MDSCs, M2 macrophages, and/or Tregs mediated by cytokines and chemokines such as MCP1, VEGF, IL-6, IL-10 secreted from the indolent dormant cells, and (ii) the engagement of immune checkpoint molecules such as a PD-1/PD-L1 pathway. The IFN-γ released from tumor-reactive T cells is a major factor that induces/upregulates the expression of PD-L1 on indolent tumor cells, and result in the engagement of the immune checkpoint pathway.

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Conflicts of interest

The authors report no conflicts of interest.

References

Aceto N, Bardia A, Miyamoto DT, et al. (2014) Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. Cell, 158(5), 1110–1122. doi:10.1016/j.cell.2014.07.013

Aguirre-Ghiso JA. (2007). Models, mechanisms and clinical evidence for cancer dormancy. Nat Rev Cancer, 7(11), 834–846. doi:nrc2256[pii]

Ali FR, Lear JT. (2012). Melanoma in organ transplant recipients: Incidence, outcomes and management considerations. J Skin Cancer, 404615. doi:10.1155/2012/404615

Atkins MB, Lotze MT, Dutcher JP, et al. (1999). High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: Analysis of 270 patients treated between 1985 and 1993. J Clin Oncol Off J Am Soc Clin Oncol, 17(7), 2105–2116.

Beatty GL, Paterson Y. (2000). IFN-gamma can promote tumor evasion of the immune system in vivo by down-regulating cellular levels of an endogenous tumor antigen. J Immunol, 165(10), 5502–5508.

Bergmann C, Wild CA, Narwan M, et al. (2011). Human tumor-induced and naturally occurring treg cells differentially affect NK cells activated by either IL-2 or target cells. Eur J Immunol, 41 (12), 3564–3573. doi:10.1002/eji.201141532

Borsutzky S, Cazac BB, Roes J, Guzman CA. (2004). TGF-beta receptor signaling is critical for mucosal IgA responses. J Immunol, 173(5), 3305–3309. doi:173/5/3305 [pii]

Buell JF, Gross TG, Woodle ES. (2005). Malignancy after transplantation. Transplantation, 80(2 Suppl), S254–64. doi:00007890-200510151-00011 [pii]

Demicheli R, Miceli R, Moliterni A, et al. (2005). Breast cancer recurrence dynamics following adjuvant CMF is consistent with tumor dormancy and mastectomy-driven acceleration of the metastatic process. Ann Oncol Off J Eur Soc Med Oncol/ESMO, 16(9), 1449–1457. doi:mdi280[pii]

Dickerson EC, Davenport MS, Liu PS. (2015). Spontaneous regression of primary renal cell carcinoma following image-guided percutaneous biopsy. Clin Imaging, 39(3), 520–524. doi:10.1016/j.clinimag.2014.08.002

Dignass AU, Podolsky DK. (1993). Cytokine modulation of intestinal epithelial cell restitution: Central role of transforming growth factor beta. Gastroenterology, 105(5), 1323–1332. doi: S001650859300352X [pii]

Dunn GP, Bruce AT, Ikeda H, et al. (2002). Cancer immunoediting: From immunosurveillance to tumor escape. Nat Immunol, 3(11), 991–998.

Dunn GP, Old LJ, Schreiber RD. (2004). The three Es of cancer immunoediting. Ann Rev Immunol, 22, 329–360. doi:10.1146/annurev.immunol.22.012703.104803

Engels EA, Pfeiffer RM, Fraumeni Jr, JF, et al. (2011). Spectrum of cancer risk among US solid organ transplant recipients. JAMA, 306(17), 1891–1901. doi:10.1001/jama.2011.1592

Farrar JD, Katz KH, Windsor J, et al. (1999). Cancer dormancy. VII. A regulatory role for CD8+ T cells and IFN-gamma in establishing and maintaining the tumor-dormant state. J Immunol, 162 (5), 2842–2849.

Gerber SA, Sorensen EW, Sedlacek AL, et al. (2013). Local expression of interleukin-2 by B16 melanoma cells results in decreased tumour growth and long-term tumour dormancy. Immunology, 138(3), 280–292. doi:10.1111/imm.12037

Ghajar CM, Peinado H, Mori H, et al. (2013). The perivascular niche regulates breast tumour dormancy. Nat Cell Biol, 15(7), 807–817. doi:10.1038/ncb2767

Goding SR, Wilson KA, Xie Y, et al. (2013). Restoring immune function of tumor-specific CD4+ T cells during recurrence of melanoma. J Immunol, 190(9), 4899–4909. doi:10.4049/jimmunol.1300271

Haiqi H, Yong Z, Yi L. (2011). Transcriptional regulation of Foxp3 in regulatory T cells. Immunobiology, 216(6), 678–685. doi:10.1016/j.imbio.2010.11.002

Halliday GM, Patel A, Hunt MJ, et al. (1995). Spontaneous regression of human melanoma/nonmelanoma skin cancer: Association with infiltrating CD4+ T cells. World J Surg, 19(3), 352–358.

Howe KL, Reardon C, Wang A, et al. (2005). Transforming growth factor-beta regulation of epithelial tight junction proteins enhances barrier function and blocks enterohemorrhagic Escherichia Coli O157: H7-inducedincreased permeability. Am J Pathol, 167(6), 1587–1597. doi: S0002-9440(10)61243-6 [pii]

Jensen SM, Twitty CG, Maston LD, et al. (2012). Increased frequency of suppressive regulatory T cells and T cell-mediated antigen loss results in murine melanoma recurrence. J Immunol, 189(2), 767–776. doi:10.4049/jimmunol.1103822

Kim ST, Jeong H, Woo OH, et al. (2013). Tumor-infiltrating lymphocytes, tumor characteristics, and recurrence in patients with early breast cancer. Am J Clin Oncol, 36(3), 224–231. doi: 10.1097/COC.0b013e3182467d90

Kmieciak M, Gowda M, Graham L, et al. (2009). Human T cells express CD25 and Foxp3 upon activation and exhibit effector/memory phenotypes without any regulatory/suppressor function. J Transl Med, 7, 89-5876-7-89. doi:10.1186/1479-5876-7-89

Kmieciak M, Knutson KL, Dumur CI, Manjili MH. (2007). HER-2/Neu antigen loss and relapse of mammary carcinoma are actively induced by T cell-mediated anti-tumor immune responses. Eur J Immunol, 37(3), 675–685.

Kmieciak M, Payne KK, Idowu MO, et al. (2011). Tumor escape and progression of HER-2/Neu negative breast cancer under immune pressure. J Transl Med, 9, 35-5876-9-35.

Kmieciak M, Payne KK, Wang XY, Manjili MH. (2013). IFN-gamma ralpha is a key determinant of CD8+ T cell-mediated tumor elimination or tumor escape and relapse in FVB mouse. PloS One, 8(12), e82544.

Manjili MH. (2014). The inherent premise of immunotherapy for cancer dormancy. Cancer Res, 74(23), 6745–6749.

Manjili MH, Payne KK. (2015). Prospects in cancer immunotherapy: Treating advanced stage disease or preventing tumor recurrence? Discovery Med, 19(107), 427–431.

Menon MP, Eaton KD. (2015). Spontaneous regression of non-small-cell lung cancer in AIDS after immune reconstitution. J Thoracic Oncol Off Publ Int Assoc Study Lung Cancer, 10(1), e1–2. doi: 10.1097/JTO.0000000000000236

Muto S, Owada Y, Inoue T, et al. (2015). Clinical significance of expanded Foxp3(+) Helios(-) regulatory T cells in patients with non-small cell lung cancer. Int J Oncol, 47(6), 2082–2090. doi: 10.3892/ijo.2015.3196

Payne KK, Keim RC, Graham L, et al. (2016). Tumor-reactive immune cells protect against metastatic tumor and induce immunoediting of indolent but not quiescent tumor cells. J Leukocyte Biol. doi: jlb.5A1215-580R [pii]

Penn I. (1988). Tumors of the immunocompromised patient. Ann Rev Med, 39, 63–73. doi: 10.1146/annurev.me.39.020188.000431

Planchon SM, Martins CA, Guerrant RL, Roche JK. (1994). Regulation of intestinal epithelial barrier function by TGF-beta 1. Evidence for its role in abrogating the effect of a T cell cytokine. J Immunol, 153(12), 5730–5739.

Printz C. (2001). Spontaneous regression of melanoma may offer insight into cancer immunology. J Natl Cancer Inst, 93(14), 1047–1048.

Roers A, Siewe L, Strittmatter E, et al. (2004). T cell-specific inactivation of the interleukin 10 gene in mice results in enhanced T cell responses but normal innate responses to lipopolysaccharide or skin irritation. J Exp Med, 200(10), 1289–1297. doi:jem.20041789 [pii]

Sakaguchi S, Yamaguchi T, Nomura T, Ono M. (2008). Regulatory T cells and immune tolerance. Cell, 133(5), 775–787. doi:10.1016/j.cell.2008.05.009

Sayour EJ, McLendon P, McLendon R, et al. (Apr, 2015). Increased proportion of FoxP3+ regulatory T cells in tumor infiltrating lymphocytes is associated with tumor recurrence and reduced survival in patients with glioblastoma. Cancer Immunol Immunother CII, 64(4), 419–427. doi:10.1007/s00262-014-1651-7

Suzuki H, Onishi H, Morisaki T, et al. (2013). Intratumoral FOXP3+VEGFR2+ regulatory T cells are predictive markers for recurrence and survival in patients with colorectal cancer. Clin Immunol (Orlando, Fla.), 146(1), 26–33. doi:10.1016/j.clim.2012.10.007

Tokunaga E, Okano S, Nakashima Y, et al. (2014). Spontaneous regression of breast cancer with axillary lymph node metastasis: A case report and review of literature. Int J Clin Exp Pathol, 7(7), 4371–4380.

Vajdic CM, McDonald SP, McCredie MR, et al. (2006). Cancer incidence before and after kidney transplantation. JAMA, 296(23), 2823–2831. doi:296/23/2823 [pii]

Vajdic CM, van Leeuwen MT. (2009). Cancer incidence and risk factors after solid organ transplantation. Int J Cancer, 125(8), 1747–1754. doi:10.1002/ijc.24439

Wang J, Ioan-Facsinay A, van der Voort EI, et al. (2007). Transient expression of FOXP3 in human activated nonregulatory CD4+ T Cells. Eur J Immunol, 37(1), 129–138. doi:10.1002/eji.200636435

Whiteside TL, Letessier E, Hirabayashi H, et al. (1993). Evidence for local and systemic activation of immune cells by peritumoral injections of interleukin 2 in patients with advanced squamous cell carcinoma of the head and neck. Cancer Res, 53(23), 5654–5662.

Ziegler SF. (2007). FOXP3: Not just for regulatory T cells anymore. Eur J Immunol, 37(1), 21–23. doi:10.1002/eji.200636929

April 10, 2017

Dear Kyle Payne,

I am very pleased to invite you to speak at a special session to feature investigators with the highest JLB citations in 2016 to be acknowledged at the 2017 Annual Meeting of the *Society for Leukocyte Biology (SLB.)* This meeting entitled "*Leukocyte Memory: Health and Disease*" will be held October 5-7, 2017, in Vancouver, BC, Canada at the Westin Bayshore. Based on your article, "*Tumor-reactive immune cells protect against metastatic tumor and induce immunoediting of indolent but not quiescent tumor cells*" that was highly cited, you are invited to speak about this topic in general in the following session.

Concurrent Session #8: Best of *Journal of Leukocyte Biology*
Date/Time: Saturday, October 7, 10:45am
Total Talk Duration: 20 min. (please allow approximately 7min. for questions)

Invited speakers will be provided with the following:

- ***Up to*** $500 USD airfare travel reimbursement for speakers coming from the USA & Canada, or **up to** $1000 USD airfare travel reimbursement for international travel. The meeting will cover the **lowest available economy fare**, advanced purchase, between home city and meeting destination.
- Three nights hotels accommodations at single room rate (rooms will be billed to the master account with no need for receipts or reimbursements)
- Waived registration to the conference

We sincerely hope that you will be able to accept our invitation. If you have any questions, please do not hesitate to contact me or SLB's Executive Director, Jennifer Holland (jholland@leukocytebiology.org).

After reviewing this, please inform our meeting manager, Kendra LaDuca (meetings@leukocytebiology.org) of your decision no later than April 17, 2017. If you accept this invitation, please confirm to us that you will plan to attend/participate in the entire conference for the benefit of all junior attendees, and that in the event your plans change and you cannot attend, you will advise the program committee immediately so that an alternative speaker can be arranged. Please note that support for travel and accommodations is fixed to the amount stated above.

Thank you in advance for your consideration and we hope you can join us in Vancouver!

Sincerely,

Luis Montaner
JLB Editor in Chief
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GUEST EDITORS' INTRODUCTION

Immune Regulatory Function of Tregs

Masoud H. Manjili and Kyle K. Payne

Regulatory CD4+ T cells (Tregs) have become increasingly appreciated in their role of facilitating immunological tolerance during homeostasis and during the diseased state. A general understanding of the literature characterizes Tregs as key elements of the immune system involving in the suppression of immune responses against cancer or infectious diseases, as well as the prevention of autoimmune diseases. However, the immune regulatory role of Tregs goes beyond this simple suppressive function, since, for example, Tregs actively contribute to the maintenance of intestinal epithelial cell homeostasis by producing TGF-β, which in turn helps B cells produce IgA. Therefore, topics that were selected in this thematic issue cover the pleiotropic functions of Tregs during homeostasis and disease, which we hope will contextualize these cells within the broader immunological terrain. This thematic issue is meant to provide an overview of the contribution of Tregs during subclinical diseases or cancer dormancy as well as clinical diseases including cancer and autoimmunity. Authors were selected based on their research interests and accomplishments in the area of cellular immunology, with a focus on T cell biology. To this end, the contribution of Fabian Benencia of Ohio University, an expert on antigen presentation during the activation or suppression of T cells (Benencia et al., 2014), focuses on the role of Tregs in ovarian cancer (Singh et al., 2016), and outlines experimental approaches to impair their immunosuppressive function.

Paula Bos of Virginia Commonwealth University (VCU) is an expert on the interaction of Tregs with the tumor microenvironment. She investigated the role of Tregs during tumor progression during her postdoctoral training in the laboratory of Dr. Rudensky (Bos et al., 2013). In the review provided by Dr. Bos, we are introduced to non-classical functions of Tregs. Beyond the more classical role of Tregs as suppressors of immunity, these cells have also been shown to be contributors to tissue remodeling and repair, and have been demonstrated to exhibit immune-independent functions, such as angiogenesis. Dr. Bos discusses such alternative mechanisms by which Tregs may contribute to tumor progression (Bos, 2016).

Nejat Egilmez of the University of Louisville is an expert in the area of Th1 immune responses and modulation of Tregs during immunotherapy (Li et al., 2015). In their review, Li and Egilmez discuss the ontogeny of tumor-associated Tregs. The well-established and critical contribution of Tregs to immune suppression in the tumor microenvironment is discussed. The authors importantly consider that information regarding the origin and population dynamics of Tregs remains limited. The central question brought forward in this review is the relative contribution of thymic Tregs and peripheral Tregs to the total tumor Treg population, and the mechanisms underlying the prevalence of each population in tumors. Therefore, the ontogeny of tumor-associated Tregs is discussed in this review (Li and Egilmez, 2016).

B.J. Monzavi-Karbassi of the University of Arkansas for Medical Sciences is an expert in the area of cancer vaccines (Monzavi-Karbassi et al., 2007) whose work provides an insight into the role of Tregs during vaccination. In a contribution of original work, Monzavi-Karbassi et al. investigated the effect of modulation of the expression of tumor-associated antigens in influencing the immunogenicity of a cell-based vaccination strategy. Interestingly, they observed that crude tumor-secreted antigens activated Tregs and induced their suppressive potential. This suggests that tumor-associated antigens can be enriched using their glycan expression pattern to weaken immune suppression and to improve antitumor immune responses (Monzavi-Karbassi et al., 2016).

Masoud Manjili of VCU Massey Cancer Center is a tumor immunologist whose research program is focused on immunotherapy of breast cancer and targeting tumor dormancy while overcoming immune suppressor cells (Manjili, 2014; Payne et al., 2016). In their review, Manjili and Butler discuss the poorly understood concept of tumor cell dormancy in the context of immune-mediated maintenance, as well as escape and subsequent recurrence. Given this poorly defined nature of immune responses in the setting of tumor dormancy, the authors' contribution provides a well-timed review of the literature related to the role of Tregs to the maintenance of tumor dormancy and/or recurrence (Manjili and Butler, 2016).

Kyle Payne of the Wistar Institute is an expert in the cellular crosstalk of the tumor immuno-environment, and modulation of T cell responses as well as immune suppressor cells (Payne et al., 2016; Payne et al., 2013). In his review, Dr. Payne discusses the crosstalk Tregs establish with myeloid cells in the tumor microenvironment, and also discusses the emerging appreciation of γδ-T cells as atypical regulators of antitumor immunity (Payne, 2016).

Qingguo Ruan is an expert in the field of immune regulation and the pathogenesis of autoimmune disease (Ruan et al., 2011). The original work by Wang and others from the laboratory of Dr. Ruan (Wang et al., 2016) investigates the requirement of the NF-κB family transcription factor, c-Rel, in the *in vivo* generation of peripherally induced Tregs. The data presented by the authors suggest that c-Rel may play distinct roles in regulating the development of peripherally induced Tregs within diverse tissue microenvironments (Wang et al., 2016).

The original work by Sznurkowska et al. investigates regulatory T cells in children with inflammatory bowel disease (IBD). The authors hypothesized that defective immune regulation leads to pathological immune responses directed against gut flora at the onset of IBD, therefore they describe a study which quantified Tregs in these patients in order to identify possible correlations between the presence of regulatory T cell and the pathology of IBD (Sznurkowska et al., 2016).

Anthony Vella of the University of Connecticut is an expert in the area of dual co-stimulation of T cells, and modulation of Tregs, both CD4+ and CD8+ Tregs (St Rose et al., 2013). Wang and Vella summarize the current knowledge on the roles of Tregs during cancer development, as well as the underlying cellular and molecular mechanisms in their review. They discuss the dual role of Tregs in functioning for the development, progression, and treatment of cancers, in which evidence is cited for their suppressive function against antitumor immunity, as well as the ability of Tregs to act directly on transformed epithelial cells to exert opposing effects during cancer development (Wang and Vella, 2016).

Xingxing Zang of the Albert Einstein College of Medicine is an expert in T cell biology and the mechanisms by which co-stimulation and co-inhibition regulate T cells (Zang et al., 2016). The review contributed from Dr. Zang's laboratory by Liu et al. discusses the role of co-stimulatory and co-inhibitory signals in being key mechanistic contributors to the regulation of adaptive immunity, and, further, discusses the recent progress in delineating the roles of co-stimulatory and co-inhibitory signals in the context of Tregs (Liu et al., 2016).

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References

Benencia F, Muccioli M, Alnaeeli M. (2014). Perspectives on reprograming cancer-associated dendritic cells for anti-tumor therapies. Front Oncol, 4, 72. doi:10.3389/fonc.2014.00072.

Bos PD. (2016). TREG cells in cancer: Beyond classical immunological control. Immunol Invest, 45, 721–728.

Bos PD, Plitas G, Rudra D, et al. (2013). Transient regulatory T cell ablation deters oncogene-driven breast cancer and enhances radiotherapy. J Exp Med, 210(11), 2435–2466. doi:10.1084/jem.20130762.

Li Q, Egilmez NK. (2016). Ontogeny of tumor-associated CD4+CD25+Foxp3+ T-regulatory cells. Immunol Invest, 45, 729–745.

Li Q, Virtuoso LP, Anderson CD, Egilmez NK. (2015). Regulatory rebound in IL-12-treated tumors is driven by uncommitted peripheral regulatory T cells. J Immunol (Baltimore, MD: 1950), 195 (3), 1293–1300. doi:10.4049/jimmunol.1403078.

Liu W, Almo SC, Zang X. (2016). Co-stimulate or co-inhibit regulatory T cells, which side to go? Immunol Invest, 45, 813–831. doi: 10.1080/08820139.2016.1186690.

Manjili MH. (2014). The inherent premise of immunotherapy for cancer dormancy. Cancer Res, 74 (23), 6745–6749.

Manjili MH, Butler SE. (2016). Role of Tregs in cancer dormancy or recurrence. Immunol Invest, 45, 759–766.

Monzavi-Karbassi B, Hennings LJ, Artaud C, et al. (2007). Preclinical studies of carbohydrate mimetic peptide vaccines for breast cancer and melanoma. Vaccine, 25(16), 3022–3031. doi: S0264-410X(07)00039-4.

Monzavi-Karbassi B, Jousheghany F, Kieber-Emmons T. (2016). Tumor-associated glycans and Tregs in immunogenicity of an autologous cell-based vaccine. Immunol Invest, 45, 746–758.

Payne KK. (2016). Lymphocyte-mediated immune regulation in health and disease: The Treg and γδ T cell co-conspiracy. Immunol Invest, 45, 767–775.

Payne KK, Keim RC, Graham L, et al. (2016). Tumor-reactive immune cells protect against metastatic tumor and induce immunoediting of indolent but not quiescent tumor cells. J Leukoc Biol, 100(3), 625–35. doi:jlb.5A1215–580R.

Payne KK, Zoon CK, Wan W, et al. (2013). Peripheral blood mononuclear cells of patients with breast cancer can be reprogrammed to enhance anti-HER-2/Neu reactivity and overcome myeloid-derived suppressor cells. Breast Cancer Res Treat, 142(1), 45–57.

Ruan Q, Wang T, Kameswaran V, et al. (2011). The microRNA-21-PDCD4 axis prevents type 1 diabetes by blocking pancreatic beta cell death. Proc Nat Acad Sci USA, 108(29), 12030–12035. doi:10.1073/pnas.1101450108.

Singh M, Loftus T, Webb E, Benencia F. (2016). Minireview: Regulatory T cells and ovarian cancer. Immunol Invest, 45, 712–720. doi:10.1080/08820139.2016.1186689.

St Rose MC, Taylor RA, Bandyopadhyay S, et al. (2013). CD134/CD137 dual costimulation-elicited IFN-gamma maximizes effector T-cell function but limits Treg expansion. Immunol Cell Biol, 91 (2), 173–183. doi:10.1038/icb.2012.74.

Sznurkowska K., Żawrocki A., Sznurkowski J., et al. (2016). Peripheral and intestinal T-regulatory cells are upregulated in children with inflammatory bowel disease at onset of disease. Immunol Invest, 45, 787–796.

Wang K, Vella AT. (2016). Regulatory T cells and cancer: A two-sided story. Immunol Invest, 45, 797–812.

Wang T, Shi W, Fan T, et al. (2016). c-Rel is required for the induction of pTregs in the eye but not in the gut mucosa. Immunol Invest, 45, 776–786. doi:10.3109/08820139.2016.1172639.

Zang X, Jiang Y, Li X, et al. (2016). Characteristics of CD4+CD25+Foxp3+ regulatory T cells in patients with multiple organ dysfunction syndrome. Exp Ther Med, 11(5), 1908–1912. doi:10.3892/etm.2016.3157.