JCI The Journal of Clinical Investigation

Heavy LIFting: tumor promotion and radioresistance in NPC

Micah Luftig

J Clin Invest. 2013;123(12):4999-5001. https://doi.org/10.1172/JCI73416.

Commentary

The epithelial-derived nasopharyngeal carcinoma (NPC) is a rare tumor in most of the world; however, it is common in southern China, northern Africa, and Alaska. NPC is often left undiagnosed and untreated until a late stage of disease. Furthermore, while radiation therapy is effective against this tumor, local recurrence due to radioresistance is an important clinical problem. In this issue, Liu et al. report on their identification of the IL-6 family cytokine leukemia inhibitory factor (LIF) as a serum predictor of local NPC recurrence following radiation therapy. The authors developed this initial finding to discover a role for the LIF/LIFR/mTORC1 signaling axis in NPC tumor cell growth as well as radioresistance.

Address correspondence to: Katalin Susztak, Room 405B Clinical Research Building, 415 Curie Boulevard, Philadelphia, Pennsylvania 19104, USA. Phone: 215.898.2009; Fax: 215.898.0189; E-mail: ksusztak@mail.med.upenn.edu.

1. D'Agati VD, Kaskel FJ, Falk RJ. Focal segmental glomerulosclerosis. *N Engl J Med*. 2011; 365(25):2398–2411.
2. Reiser J, et al. TRPC6 is a glomerular slit diaphragm-associated channel required for normal renal function. *Nat Genet*. 2005;37(7):739–744.
3. Winn MP, et al. A mutation in the TRPC6 cation channel causes familial focal segmental glomerulosclerosis. *Science*. 2005;308(5729):1801–1804.
4. Kestilä M, et al. Positionally cloned gene for a novel glomerular protein — nephrin — is mutated in congenital nephrotic syndrome. *Mol Cell*. 1998; 1(4):575–582.
5. Brown EJ, et al. Mutations in the formin gene INF2 cause focal segmental glomerulosclerosis. *Nat Genet*. 2010;42(1):72–76.
6. George B, Holzman LB. Signaling from the podocyte intercellular junction to the actin cytoskeleton. *Semin Nephrol*. 2012;32(4):307–318.
7. Wiggins RC. The spectrum of podocytopathies: a unifying view of glomerular diseases. *Kidney Int*. 2007;71(12):1205–1214.
8. Susztak K, Raff AC, Schiffer M, Böttinger EP. Glucose-induced reactive oxygen species cause apoptosis of podocytes and podocyte depletion at the onset of diabetic nephropathy. *Diabetes*. 2006;55(1):225–233.
9. Niranjan T, et al. The Notch pathway in podocytes plays a role in the development of glomerular disease. *Nat Med*. 2008;14(3):290–298.
10. Gödel M, et al. Role of mTOR in podocyte function and diabetic nephropathy in humans and mice. *J Clin Invest*. 2011;121(6):2197–2209.
11. Hildebrandt F, et al. A systematic approach to mapping recessive disease genes in individuals from outbred populations. *PLoS Genet*. 2009; 5(1):e1000353.
12. Ashraf S, et al. *ADCK4* mutations promote steroid-resistant nephrotic syndrome through CoQ_{10} biosynthesis disruption. *J Clin Invest*. 2013; 123(12):5179–5189.
13. Diomedi-Camassei F, et al. COQ2 nephropathy: a newly described inherited mitochondriopathy with primary renal involvement. *J Am Soc Nephrol*. 2007; 18(10):2773–2780.
14. Heeringa SF, et al. COQ6 mutations in human patients produce nephrotic syndrome with sensorineural deafness. *J Clin Invest*. 2011;121(5):2013–2024.
15. López LC, et al. Leigh syndrome with nephropathy and CoQ10 deficiency due to decaprenyl diphosphate synthase subunit 2 (PDSS2) mutations. *Am J Hum Genet*. 2006;79(6):1125–1129.
16. Saiki R, et al. Coenzyme Q10 supplementation rescues renal disease in Pdss2kd/kd mice with mutations in prenyl diphosphate synthase subunit 2. *Am J Physiol Renal Physiol*. 2008; 295(5):F1535–F1544.
17. Sourris KC, et al. Ubiquinone (coenzyme Q10) prevents renal mitochondrial dysfunction in an experimental model of type 2 diabetes. *Free Radic Biol Med*. 2012;52(3):716–723.
18. Persson MF, et al. Coenzyme Q10 prevents GDP-sensitive mitochondrial uncoupling, glomerular hyperfiltration and proteinuria in kidneys from db/db mice as a model of type 2 diabetes. *Diabetologia*. 2012;55(5):1535–1543.

Heavy LIFting: tumor promotion and radioresistance in NPC

Micah Luftig

Department of Medical Genetics and Microbiology, School of Medicine, Duke University, Durham, North Carolina, USA.

The epithelial-derived nasopharyngeal carcinoma (NPC) is a rare tumor in most of the world; however, it is common in southern China, northern Africa, and Alaska. NPC is often left undiagnosed and untreated until a late stage of disease. Furthermore, while radiation therapy is effective against this tumor, local recurrence due to radioresistance is an important clinical problem. In this issue, Liu et al. report on their identification of the IL-6 family cytokine leukemia inhibitory factor (LIF) as a serum predictor of local NPC recurrence following radiation therapy. The authors developed this initial finding to discover a role for the LIF/LIFR/mTORC1 signaling axis in NPC tumor cell growth as well as radioresistance.

NPC: a rare and difficult to diagnose epithelial cell cancer

Nasopharyngeal carcinoma (NPC) is a squamous-cell tumor that affects the epithelial cell lining of the nasopharynx. NPC is a rare tumor throughout the world, but it occurs with increased frequency in Southeast Asia and is tightly linked to EBV infection. While NPC can be cured by radiation therapy if diagnosed and treated early, often this cancer is not recognized until it has progressed to an advanced stage. Furthermore, approximately 20% of NPC patients have local recurrence following radiation (1). Indeed, a common cause of local recurrence and poor survival in NPC is radioresistance. While new imaging approaches have improved diagnosis and survival rates, new approaches to identifying biomarkers that predict local recurrence will be important in mitigating NPC disease burden and mortality.

Identification of serum biomarkers for NPC local recurrence

A minimally invasive approach to screening NPC patients would be to identify molecules secreted from the tumor environment into the blood that could be used as clinically predictive biomarkers. Therefore, Liu et al. screened the serum of NPC patients with local recurrence and compared it with serum from those who had gone into remission after radiation therapy (2). A panel of 20 cytokines was assayed, and a small group that included leukemia inhibitory factor (LIF), CXCL9, IL-10, IL-6, and SCF was among those differentially elevated in patients with local recurrence. Of the cytokines assayed, LIF was the most markedly different between NPC patients that responded to radiation therapy and those with local recurrence; therefore, LIF was further studied for its role in NPC pathogenesis and radioresistance.

Impressively, Liu and colleagues determined that LIF serum levels alone were predictive of NPC compared with healthy individuals (2). Furthermore, NPC patients with the highest levels of LIF were more likely to have local recurrence following radiation therapy; however, LIF levels were not predictive of either metastasis-free or overall survival. The authors also presented compelling immunohistochemical evidence that LIF levels in the tumor environment were higher than in normal tissues. Additionally, both LIF and the LIF receptor (LIFR) were overexpressed in NPC tumors as compared with adjacent tissue. These clinical observations suggested that LIF actually plays a role in NPC tumorigenesis rather than simply serving as a biomarker of local recurrence.

Conflict of interest: The author has declared that no conflict of interest exists.

Citation for this article: *J Clin Invest.* 2013; 123(12):4999–5001. doi:10.1172/JCI73416.

The LIF/LIFR interaction promotes NPC tumor growth and radioresistance

The authors next examined the role of LIF signaling in NPC tumor growth and radioresistance. LIF is a member of a family of IL-6–related cytokines that activate cell signaling pathways through both a unique and a common receptor, LIFR and glycoprotein 130 (gp130), respectively (3). LIF is known to activate the JAK/STAT3, PI3K, and ERK1/2 pathways to regulate cell growth. Recent evidence has implicated increased LIF signaling in a number of cancers including pancreatic (4), breast (5), glioblastoma (6), and thyroid (7) cancers. Therefore, strong rationale existed to investigate LIF as a growth-promoting factor in NPC tumorigenesis.

Liu et al. found that exogenous introduction of LIF signaled through LIFR to stimulate mTORC1-dependent phosphorylation of p70S6K and downstream STAT3 and ERK activation in NPC cells in culture (see Figure 9 of ref. 2). This signaling pathway was critical for NPC cell growth as well as growth of NPC xenografts in immune-deficient mice. Indeed, both soluble LIFR and rapamycin treatment prevented signaling through mTORC1 in these cells and inhibited tumor cell growth.

Follow-up immunohistochemical analysis in locally recurrent NPC tumor tissue indicated a strong upregulation and correlation of LIF, LIFR, and mTORC1 pathway activation markers. These data supported the notion that LIF may play a role in radioresistance. Indeed, the authors found that exogenous LIF could promote survival of NPC cell lines in culture and in xenograft studies following low-dose γ irradiation. Furthermore, LIF directly antagonized irradiation-mediated DNA damage response signaling, suggesting an acute mechanism in radioresistance.

Connecting EBV to LIF in NPC pathogenesis

It has long been appreciated that the ubiquitous human herpesvirus EBV is clonally present as a latent infection in nearly all NPC cases (8, 9). The virus expresses a restricted form of latency called latency II, in which the latent membrane proteins LMP1 and LMP2A are expressed along with one nuclear antigen EBNA1 and a set of viral noncoding RNAs (EBERs and BART miRNAs). The expression of LMP1 and LMP2A has been shown to constitutively activate the NF-κB (10) and PI3K pathways (11), among others, in epithelial cells and in NPC tissues (12, 13). In this study, the authors demonstrated that the viral LMP1 protein, through the NF-κB pathway, upregulated *LIF* mRNA and protein levels (see Figure 9 of ref. 2). Therefore, EBV infection and subsequent LMP1 expression in NPC tumor cells may explain the elevated LIF levels that contribute to disease pathogenesis and radioresistance.

Future questions and clinical opportunities

As expected in any important scientific study, Liu et al. (2) raise additional questions to be answered. These studies provide potential clinical opportunities for NPC, primarily in predicting recurrence following radiation therapy. The finding of increased LIF and LIFR expression in NPC tumors is compelling; however, it remains to be determined which cells in the tumor microenvironment produce the LIF that promotes tumor growth and radioresistance. Indeed, the authors provide evidence that ionizing radiation increases LIF levels, suggesting that stromal cells or immune cells within the tumor microenvironment are activated upon irradiation to produce LIF. Increased LIF in the microenvironment would then promote the growth and perhaps radioresistance of a population of tumor cells with increased LIFR levels. It is also possible, and likely, that the LIF/LIFR signaling pathway collaborates with other signaling pathways to drive tumor growth and radioresistance.

While the activity of LIF signaling through mTORC1 appears to be critical for NPC tumor growth, the collaboration between LIF/LIFR and the EBV latent membrane proteins will be important to assess in the future. Notably, LMP1 activation of the NF-κB pathway (10) and LMP2A-mediated activation of the PI3K/AKT pathway (11) may coordinate with the LIF/LIFR signaling pathway to promote tumor cell proliferation, survival, and possibly radioresistance. Indeed, recent studies suggest that the viral LMP1 protein can modulate the host DNA damage response (14, 15), which indicates collaboration between EBV and LIF for promoting radioresistance of NPC tumors following therapy.

An interesting, complementary component of LIF signaling that might be important for radioresistance is the Hippo/YAP pathway. This LIF/Yap axis has recently been implicated in mouse embryonic stem cell self renewal (16) and predicts a possible role for LIF signaling in cancer stem cell–like behavior (17). Because radioresistant cancer cells exhibit stem cell–like qualities (18), it is possible that LIF stimulation of Hippo/YAP may indeed contribute to NPC radioresistance. This exciting connection certainly warrants further investigation; it will provide insight not only into the mechanism of LIF-mediated radioresistance, but also probe the importance of stemness in the setting of NPC. Elucidation of this connection could ultimately contribute to new therapeutic targets aimed at preventing NPC recurrence.

Finally, this study addresses the question, What is a good serum biomarker for NPC recurrence and survival? Unfortunately, LIF levels alone were not predictive of overall or metastasis-free survival in NPC, despite correlating well with recurrence-free survival. Intriguingly, Liu et al. found a set of additional cytokines, including CXCL9, IL-10, IL-6, and SCF, that were also predictive of recurrence-free survival. Perhaps this cluster of cytokines together with LIF, EBV plasma viral load, or IgA levels (19) will have better predictive value for NPC survival and serve as a tool for identifying patients that fail to respond to radiation or chemotherapy. Identification of NPC prognosis-associated biomarkers remains a critically important area of research in the field. While imaging approaches to assess tumor recurrence have improved dramatically (1), a robust serological test to stratify or complement these approaches would provide great clinical benefit. Further analysis is warranted on larger numbers of patients in distinct NPC cohorts to address the potential value of LIF or related cytokines in predicting NPC progression.

In summary, the work of Liu et al. (2), along with other studies, has nicely highlighted how biomarker discovery can fuel the characterization of cell signaling pathways with potential for therapeutic relevance and mechanistic insight in tumor progression and resistance to standard therapy. This work will likely open the door for future studies focused on the role of the LIF signaling pathway in radioresistance of NPC and other tumors.

Acknowledgments

M. Luftig is supported by NIH grant 1R01-CA140337 and American Cancer Society grant RSG-13-228-01-MPC.

Address correspondence to: Micah Luftig, Duke University School of Medicine, 424 CARL Building, DUMC Box 3054, Durham, North Carolina 27710, USA. Phone: 919.668.3091; Fax: 919.681.8979; E-mail: micah.luftig@duke.edu.

1. Suárez C, Rodrigo JP, Rinaldo A, Langendijk JA, Shaha AR, Ferlito A. Current treatment options for recurrent nasopharyngeal cancer. *Eur Arch Otorhinolaryngol*. 2010;267(12):1811–1824.
2. Liu S-C, et al. Leukemia inhibitory factor promotes nasopharyngeal carcinoma progression and radioresistance. *J Clin Invest*. 2013;123(12):5269–5283.
3. Mathieu ME, et al. LIF-dependent signaling: new pieces in the Lego. *Stem Cell Rev*. 2012;8(1):1–15.
4. Kamohara H, Ogawa M, Ishiko T, Sakamoto K, Baba H. Leukemia inhibitory factor functions as a growth factor in pancreas carcinoma cells: Involvement of regulation of LIF and its receptor expression. *Int J Oncol*. 2007;30(4):977–983.
5. Estrov Z, et al. Leukemia inhibitory factor binds to human breast cancer cells and stimulates their proliferation. *J Interferon Cytokine Res*. 1995;15(10):905–913.
6. Peñuelas S, et al. TGF-β increases glioma-initiating cell self-renewal through the induction of LIF in human glioblastoma. *Cancer Cell*. 2009;15(4):315–327.
7. Arthan D, Hong SK, Park JI. Leukemia inhibitory factor can mediate Ras/Raf/MEK/ERK-induced growth inhibitory signaling in medullary thyroid cancer cells. *Cancer Lett*. 2010;297(1):31–41.
8. Nonoyama M, Pagano JS. Homology between Epstein-Barr virus DNA and viral DNA from Burkitt's lymphoma and nasopharyngeal carcinoma determined by DNA-DNA reassociation kinetics. *Nature*. 1973;242(5392):44–47.
9. Raab-Traub N, Flynn K. The structure of the termini of the Epstein-Barr virus as a marker of clonal cellular proliferation. *Cell*. 1986;47(6):883–889.
10. Miller WE, Cheshire JL, Baldwin AS, Baldwin AS Jr, Raab-Traub N. The NPC derived C15 LMP1 protein confers enhanced activation of NF-κB and induction of the EGFR in epithelial cells. *Oncogene*. 1998;16(14):1869–1877.
11. Scholle F, Bendt KM, Raab-Traub N. Epstein-Barr virus LMP2A transforms epithelial cells, inhibits cell differentiation, and activates Akt. *J Virol*. 2000;74(22):10681–10689.
12. Shair KH, Raab-Traub N. Transcriptome changes induced by Epstein-Barr virus LMP1 and LMP2A in transgenic lymphocytes and lymphoma. *MBio*. 2012;3(5):e00288-12.
13. Dawson CW, Port RJ, Young LS. The role of the EBV-encoded latent membrane proteins LMP1 and LMP2 in the pathogenesis of nasopharyngeal carcinoma (NPC). *Semin Cancer Biol*. 2012;22(2):144–153.
14. Liu MT, et al. Epstein-Barr virus latent membrane protein 1 induces micronucleus formation, represses DNA repair and enhances sensitivity to DNA-damaging agents in human epithelial cells. *Oncogene*. 2004;23(14):2531–2539.
15. Gruhne B, Sompallae R, Masucci MG. Three Epstein-Barr virus latency proteins independently promote genomic instability by inducing DNA damage, inhibiting DNA repair and inactivating cell cycle checkpoints. *Oncogene*. 2009;28(45):3997–4008.
16. Tamm C, Böwer N, Annerén C. Regulation of mouse embryonic stem cell self-renewal by a Yes-YAP-TEAD2 signaling pathway downstream of LIF. *J Cell Sci*. 2011;124(pt 7):1136–1144.
17. Zeng Q, Hong W. The emerging role of the hippo pathway in cell contact inhibition, organ size control, and cancer development in mammals. *Cancer Cell*. 2008;13(3):188–192.
18. Bao S, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*. 2006;444(7120):756–760.
19. Chien YC, et al. Serologic markers of Epstein-Barr virus infection and nasopharyngeal carcinoma in Taiwanese men. *N Engl J Med*. 2001;345(26):1877–1882.

Tuning mTOR activity for immune balance

Kai Yang and Hongbo Chi

Department of Immunology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA.

The mTOR pathway orchestrates diverse physiological processes, including T cell functions and fate decisions; however, the regulation of mTOR-dependent T cell differentiation remains elusive. In this issue, Park et al. examine the role of TSC1, an mTOR signaling regulator, in T cell differentiation and the balance between T cell–mediated immunity and tolerance. They found that enhanced mTOR activity in *Tsc1*-deficient T cells promotes Th1 and Th17 differentiation, leading to increased intestinal inflammation in murine colitis. *Tsc1*-deficient Tregs had impaired suppressive activity in inflammatory conditions. These defects were associated with the acquisition of effector-like phenotypes and could be further exacerbated by concomitant loss of transcription factor Foxo3. This study highlights that TSC1-mediated control of mTOR activity impinges on the balance between immunity and tolerance by dictating effector and regulatory T cell responses.

Introduction

mTOR is an evolutionarily conserved serine/threonine kinase that couples diverse cellular and environmental cues to cell growth, proliferation, and differentiation. In mammalian cells, mTOR interacts with multiple proteins and forms two distinct complexes, mTOR complex 1 (mTORC1) and mTORC2, which exhibit distinct roles in many physiological processes (1). The scaffold proteins Raptor and Rictor are characterized as obligatory components of mTORC1 and mTORC2, respectively (Figure 1). mTORC1 activity is tightly controlled by multiple regulators. The upstream TSC complex, composed of tuberous sclerosis complex 1 (TSC1) and TSC2, inhibits mTORC1 activity by suppressing the function of Rheb (Ras homolog enriched in brain). Mutations of *TSC1* or *TSC2* are associated with hamartoma syndrome with tissue overgrowth. Furthermore, mTOR dysfunction contributes to a large number of human diseases, including cancer, obesity, type 2 diabetes, and neurodegeneration (1).

In the immune system, T cells play a pivotal role in adaptive immunity. Emerging evidence reveals that mTOR signaling impinges on multiple physiological processes of T cells, including their development, homeostasis, proliferation, and differentiation (2). Disruption of mTORC2 by ablation of Rictor impairs Notch-mediated proliferation and differentiation of pre–T cells in thymus (3, 4) and delays malignant transformation in a murine model of T cell acute lymphoblastic leukemia (T-ALL) (3). In the peripheral immune compartment, mTOR-deficient $CD4^+$ T cells are unable to differentiate into effector cells, including Th1, Th2, and Th17 cells, and instead preferentially develop into induced Tregs (5). Furthermore, Rheb/mTORC1 and mTORC2 are selectively involved in the lineage differentiation of effector T cells (6, 7). Aside from these recent advances in our understanding of the core machinery of mTOR signaling, we are also starting to appreciate the T cell–modifying effects of dysregulated mTOR activity via the genetic modulation of mTOR upstream regulators (2). Under steady state, TSC1 deficiency and the ensuing mTORC1 activation disrupt the quiescence of naive T cells, leading to defective cell survival and antibacterial immune responses (8–10). Thus, keeping mTORC1 in check is essential for the maintenance of T cell homeostasis; however, the function of

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J Clin Invest.* 2013; 123(12):5001–5004. doi:10.1172/JCI73202.