CCR frontiers IN SCIENCE

January 2004, Volume 3 Published by the Center for Cancer Research, National Cancer Institute

Contents

Immunology 1
Dissecting Lymphotoxin Functions by Using Cre-loxP Knockout Technology

Translational Research 2
Novel Roles for Chemokine Receptors in Directing Organ-selective Metastasis

Molecular Biology 4
Below Our Radar: Small RNAs Are Stealthy Regulators of Gene Expression

Cancer and Cell Biology 6
The Receptor Interacting Protein Plays an Essential Role in DNA Damage-induced NF-κB Activation

From the Director's Office 7
A New Paradigm for Multidisciplinary Collaboration: The Molecular Targets Development Program

Administrative Links 8

NATIONAL INSTITUTES OF HEALTH
DEPARTMENT OF HEALTH AND HUMAN SERVICES

IMMUNOLOGY

Dissecting Lymphotoxin Functions by Using Cre-loxP Knockout Technology

Tumanov AV, Kuprash DV, Lagarkova MA, Grivennikov SI, Abe K, Shakhov AN, Drutskaya LN, Stewart C, Chervonsky AV, and Nedospasov SA. Distinct role of surface lymphotoxin expressed by B cells in the organization of secondary lymphoid tissues. *Immunity* 17: 239-50, 2002.

Lymphotoxin (LT) was described 35 years ago as a cytotoxic factor produced by lymphocytes. In 1984 molecular cloning unexpectedly revealed its close relation to tumor necrosis factor, which was discovered by Lloyd Old and colleagues (Carswell E et al. *Proc Natl Acad Sci U S A* 72: 3666-70, 1975) because of LT's prominent antitumor activity.

The phenotypes of LTα and LTβ knockout (KO) mice highlighted the totally unpredicted role of LT in the development and maintenance of lymphoid tissues (De Togni P et al. *Science* 264: 703-7, 1994). Many immunologists were surprised that mice can live happily without lymph nodes and Peyer's patches. Subsequent studies have indicated that formation of peripheral lymphoid organs (e.g., lymph nodes) and their maintenance in adult animals are tightly regulated. The interactions between lymphocytes and the stroma of lymphoid organs are critical for these processes, and lymphocytes actively participate in creating an optimal microenvironment by sending signals to the receptors on stromal cells. One such signal involves surface LT.

An important recent application of Cre-loxP technology in dissecting LT functions *in vivo* was based on cell type-specific inactivation of LT. This specificity was desirable, because LT can be produced by several cell types and because the phenotype of LT-deficient mice is rather complex, with both embryonic and postnatal components.

In collaboration with Colin Stewart, DPhil (Cancer and Developmental Biology Laboratory) and using embryonic stem cells derived from C57/BL6 mice, Alexei Tumanov, MD, PhD, from our sister laboratory in Moscow (Engelhardt Institute of Molecular Biology) generated the LTβ "floxed" mice. We first disrupted the LTβ gene in B cells by using B cell-specific CD19-Cre deleter mice (B-LTβ KO mice) developed in the laboratory of Klaus Rajewsky, MD (Institute of Genetics, Cologne).

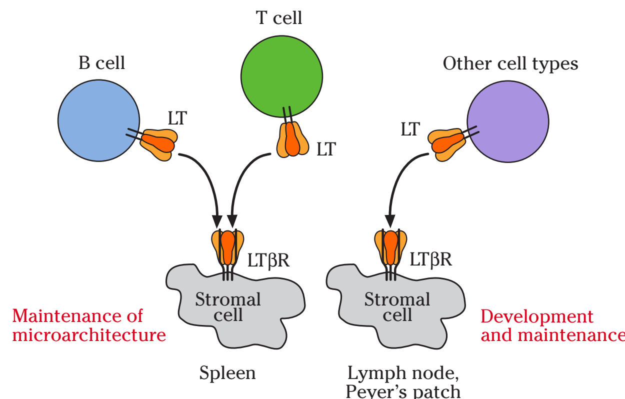

Figure 1. The distinct requirements for lymphotoxin (LT) signaling in the spleen and other peripheral lymphoid tissues. LTβR, lymphotoxin β receptor.

The B-LTβ KO mice showed the following unique phenotype. 1) All lymphoid organs were present, in clear contrast to mice with complete LTβ inactivation, which lack Peyer's patches and most lymph nodes. 2) Spleens showed profound structural defects, both in the organization of the follicles and in the marginal zone. 3) Surprisingly, peripheral lymphoid organs (e.g., lymph nodes and Peyer's patches) not only developed normally but also showed normal internal structure, including normal clusters of follicular dendritic cells. This finding argued for distinct maintenance mechanisms in different lymphoid organs. 4) Immune responses to sheep red blood cells were defective although not as poor as in mice with complete LTβ ablation.

Thus, the splenic phenotype of B-LTβ KO mice has confirmed the critical role of surface LT signaling from B lymphocytes to the stroma of lymphoid tissues in creating the optimal microenvironment needed for efficient immune response. Nevertheless, the incomplete disruption of follicular dendritic cell clusters and the retention of components of the marginal zone prompted us to consider possible contributions from other cell types.

The role of T cells was addressed next. Employing lck-Cre deleter mice from the laboratory of Junji Takeda, PhD (Osaka University), we produced T cell-specific LTβ KO mice (T-LTβ KO mice) and then double KO mice (T,B-LTβ KO mice). In contrast to B-LTβ KO mice, T-LTβ KO mice did not show any structural defects in spleen or other lymphoid tissues and developed normal immune responses. Comparison between phenotypes of B-LTβ, T,B-LTβ, and complete LTβ KO mice provided strong evidence for collaboration by T cells and implied that the critical signal also involves surface LT (Figure 1). Which specific subsets of T cells are involved remains unknown. As already indicated, the "rules" for other lymphoid organs are different.

Importantly, immune responses were more severely disturbed in T,B-LTβ KO mice than in B-LTβ KO mice and were similar to the immune responses of complete LTβ KO mice. In addition, our studies clearly implicated the essential role of surface LT produced by other cell lineages (Figure 1). It is already known that LT is expressed by so-called lymphoid tissue-initiating cells (LTIC) and that these cells are most likely responsible for the embryonic component of LT deficiency—failure to develop Peyer's patch anlagen and lymph nodes (Mebius RE. *Nat Rev Immunol* 3: 292-303, 2003). Which cells provide maintenance signals remains unknown.

In conclusion, application of Cre-mediated KO technology to study cytokine biology and function is a rewarding approach and . . . fun.

■ **Sergei A. Nedospasov, PhD, DSc**
Principal Investigator
Laboratory of Immunoregulation, Center for Cancer Research
Basic Research Program, SAIC-Frederick
NCI-Frederick, Bldg. 560/Rm. 3170
Tel: 301-846-6682
Fax: 301-846-7268
nedospas@ncifcrf.gov

■ TRANSLATIONAL RESEARCH

Novel Roles for Chemokine Receptors in Directing Organ-selective Metastasis

Murakami T, Cardones AR, Finkelstein SE, Restifo NP, Klaunberg BA, Nestle FO, Castillo SS, Dennis PA, and Hwang ST. Immune evasion by murine melanoma mediated through CC chemokine receptor-10. *J Exp Med* 198: 1337-47, 2003.

With melanoma, as with many other cancers, metastasis is the leading cause of mortality. Clinicians have long known that cancers are predisposed to metastasize to certain organs. For melanoma, these organs include the brain, lung, liver, and skin. Although mechanisms that drive preferential metastasis to one organ over another are incompletely characterized, selective expression of chemokine receptors—a large family of transmembrane G-protein–coupled receptors—by tumor cells may provide a partial explanation. Many normal cells use chemokine receptors to facilitate site-specific localization. For example, upregulation of CCR7 is essential for Langerhans cells, the professional antigen-presenting cells of the epidermis, to home to lymphatic vessels, which constitutively produce CCR7's ligand, CCL21 (Saeki H et al. *J Immunol* 162: 2472-5, 1999).

Given observations that CCR7 was upregulated in human melanoma cell lines (Wiley HE and Hwang ST, unpublished data) and that a limited repertoire of receptors—including CCR7, CXCR4, and

Although mechanisms that drive preferential metastasis to one organ over another are incompletely characterized, selective expression of chemokine receptors by tumor cells may provide a partial explanation.

CCR10—was expressed in breast and melanoma cancer lines (Müller A et al. *Nature* 410: 50-6, 2001), we hypothesized that melanoma cells might use the same receptor (CCR7) used by Langerhans cells to facilitate entry into lymphatics. B16 murine melanoma cells, which lack expression of most chemokine receptors, were transfected with CCR7. Following injection of CCR7-overexpressing B16 cells (CCR7-B16) into the footpads of mice, metastatic cells accumulated 700-fold more efficiently in draining regional lymph nodes than did control B16 cells (Wiley HE et al. *J Natl Cancer Inst* 93: 1638-43, 2001). When injected intravenously, however, CCR7-B16 cells did not accumulate in lung tissue in greater numbers than control cells did.

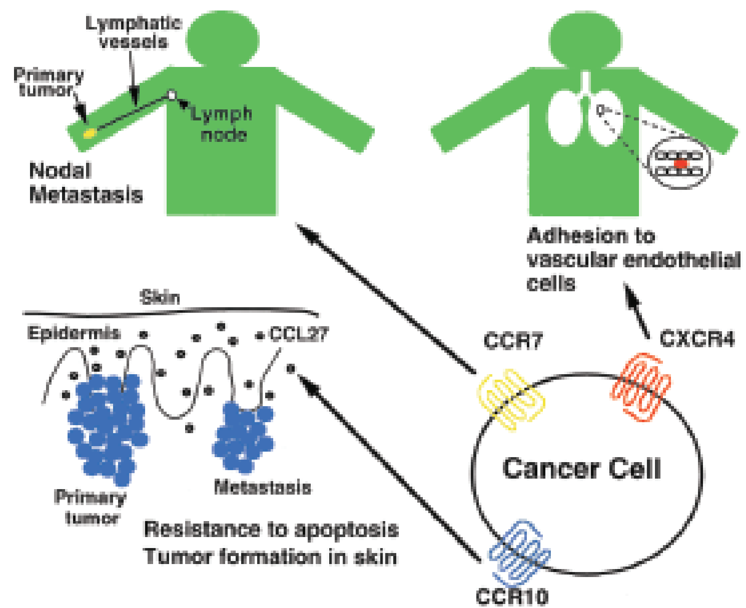

Figure 1. Roles for specific chemokine receptors in melanoma progression and metastasis. Based on observations that human melanomas express a limited subset of the known chemokine receptors, experimental results described in the text explain possible mechanisms by which these receptors may facilitate cancer dissemination and survival. The chemokine ligands for CCR10, CCR7, and CXCR4 were specifically expressed at relatively high levels in skin, lymphatic vessels and lymph nodes, and lung, respectively.

Interestingly, when CXCR4 was overexpressed in B16 cells, no increase in nodal metastasis was observed after footpad injection, although a 6- to 10-fold increase in lung accumulation of these cells was observed following intravenous inoculation (Murakami T et al. *Cancer Res* 62: 7328-34, 2002). CXCR4 activation potentially acts in multiple ways to increase the metastatic properties of tumor cells. First, in the presence of CXCL12, the ligand for CXCR4, CXCR4-B16 cells accumulated in greater numbers *in vitro* under growth factor-depleted conditions. Second, CXCR4-B16 adhered better to endothelial cells, which constitutively produce CXCL12, than did B16 cells that did not express this receptor. Subsequent studies demonstrated that activation of CXCR4 with its ligand increases the affinity of β1 integrin on the B16 cells to adhere to one of its principal ligands, an endothelial cell-adhesion molecule called VCAM-1 (Cardones AR et al. *Cancer Res* 63: 6571-7, 2003). Thus, endothelial cell-derived CXCL12 may trigger the arrest of circulating tumor cells by activating adhesion molecules such as the β1 integrin.

One of the most frequent sites of melanoma metastasis is the skin. A role for CCR10 in skin metastasis was suspected because epidermal cells (keratinocytes) in skin constitutively produce the CCR10 ligand, CCL27. The importance of CCR10 in melanoma progression was suggested by the finding that B16 cells expressing luciferase, but not CCR10, were quickly eradicated by the host immune cells in a Fas-dependent fashion following inoculation into the ear skin of mice. CCR10-B16 cells that also expressed luciferase, however, resisted immune cell-mediated death and formed progressive tumors in the skin. It is quite likely that CCR10-B16 cells inoculated in the skin were exposed to activating amounts of CCL27. To determine whether activation of CCR10 through CCL27 resulted in protection from death, CCR10-B16 cells were cross-linked *in vitro* with recombinant Fas ligand, which effectively induced cellular apoptosis. Strikingly, nearly 80 percent of cell death could be prevented if CCR10-B16 cells were exposed to CCL27 before induction of apoptosis. This finding suggests that certain chemokine receptors may effectively help cancer cells evade important killing mechanisms of immune cells, including those that are Fas mediated. Moreover, neutralizing CCL27 with antibodies prevented CCR10-B16 cells from forming tumors in the skin (Murakami T et al. *J Exp Med* 198: 1337-47, 2003).

In summary, chemokine receptors expressed by cancer cells may act to increase nodal metastasis (CCR7), increase adhesion to vascular endothelial cells (CXCR4), and allow malignant cells to evade host responses (CCR10), as depicted in Figure 1. Thus, chemokine receptors appear to play non-redundant roles in organ-selective metastasis. Preliminary evidence in animal models suggests that inhibiting the function of these receptors may decrease the ability of cancer cells to disseminate to other sites or may block their ability to survive and progress.

■ **Sam T. Hwang, MD, PhD**
Principal Investigator
Dermatology Branch
NCI-Bethesda, Bldg. 10/Rm. 12N246
Tel: 301-496-8724
Fax: 301-496-5370
hwangs@mail.nih.gov

Below Our Radar: Small RNAs Are Stealthy Regulators of Gene Expression

Repoila F, Majdalani M, and Gottesman S. Small non-coding RNAs, co-ordinators of adaptation processes in *Escherichia coli:* the RpoS paradigm. *Mol Microbiol* 48: 855-61, 2003.

The genome sequence of an organism predicts many likely regulatory proteins, but finding a new and powerful family of regulators, the non-coding RNAs (ncRNAs), is more challenging. Small ncRNAs can interact with proteins or mRNA targets to affect their activity or stability, respectively. The eukaryotic microRNAs and RNAi have recently gained a great deal of attention (*Science*, Molecule of the Year, 2002) (Storz G. *Science* 296: 1260-3, 2002). In prokaryotes such as *Escherichia coli*, up to 100 such ncRNAs have been identified and found to regulate a variety of processes. One example is the regulation of a major transcriptional regulatory protein, RpoS, by multiple small ncRNAs.

The RpoS protein of *E. coli* is a specialized sigma factor, a subunit of RNA polymerase that directs the transcription of specific promoters. The RpoS molecular switch turns on genes needed by bacteria when cell growth slows in the stationary phase and in response to many stresses. Under these conditions the amount of RpoS increases and RpoS-dependent promoters are expressed, helping the cell deal with the stress and prepare for a return to normal growth (Hengge-Aronis R. *Microbiol Mol Biol Rev* 66: 373-95, 2002). Increasing RpoS levels only when the protein is needed requires novel levels of regulation. Surprisingly, most of the regulation is not at the level of transcription; instead, regulation of translation and regulation of protein degradation play very important roles.

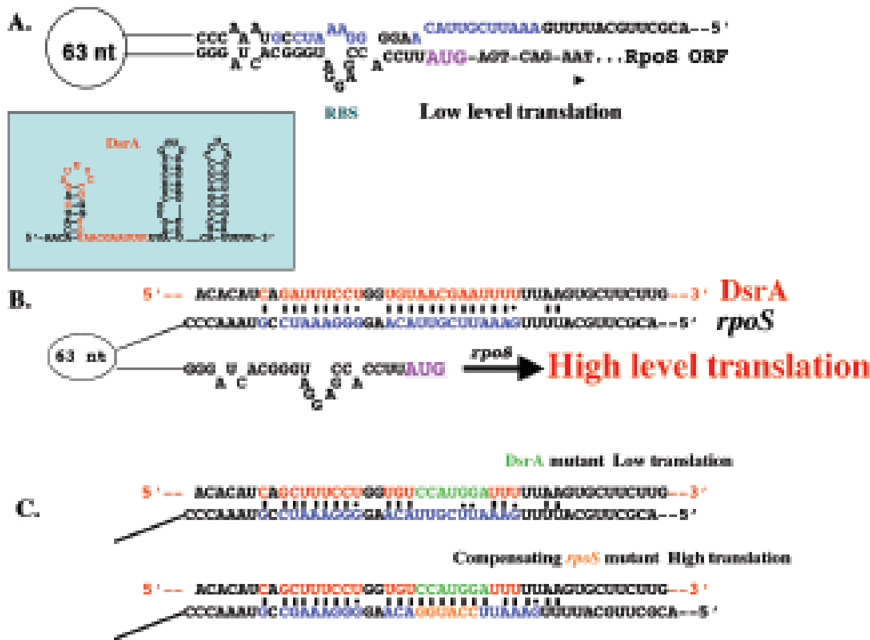

Figure 1. Model of DsrA small non-coding RNA (ncRNA) action. *A:* The mRNA of the protein RpoS forms a paired structure whereby the upstream leader loops to occlude the ribosome-binding site (RBS), resulting in a low level of RpoS translation. The stop codon is highlighted in purple. The blue nucleotides are complementary to the red nucleotides in the small ncRNA DsrA (*inset*). nt, nucleotides; ORF, open reading frame. *B:* Pairing of complementary nucleotides between DsrA small ncRNA and *rpoS* mRNA leads to a freeing of the RBS and a high level of RpoS translation. *C:* Example of a mutant used to demonstrate pairing. Mutations in the green DsrA small ncRNA result in a loss of activation of RpoS translation. Orange compensating RNA mutations introduced in the *rpoS* leader restore the pairing and a high level of translation.

Translation of RpoS is regulated by a long upstream hairpin leader RNA that folds back and occludes the ribosome-binding site (Figure 1A). When the hairpin is disrupted, translation is at a high constitutive level. How, then, is the inhibition by this leader RNA environmentally regulated? We unexpectedly found small ncRNAs play a critical regulatory role.

Two small ncRNAs, DsrA and RprA, positively regulate the translation of RpoS. DsrA and RprA both pair with the inhibitory portion of the hairpin and displace it (Figure 1B) (Majdalani N et al. *Mol Microbiol* 46: 813-26, 2002; Majdalani N et al. *Proc Natl Acad Sci U S A* 95: 12462-7, 1998). Pairing occurs *in vivo:* mutations in either the small ncRNA or the target RNA that abolish pairing also abolish stimulation of RpoS synthesis by the small ncRNA. Stimulation is restored when compensating mutations restore pairing (Figure 1C). These small ncRNAs require a protein cofactor, an RNA chaperone called Hfq, to stimulate pairing. Hfq is in the same family of proteins as eukaryotic Sm proteins, which also act as RNA chaperones for mRNA splicing (Zhang A et al. *Mol Cell* 9: 11-22, 2002).

Although similar in their mode of action, DsrA and RprA differ in many ways. Their sequences, including the region that interacts with the *rpoS* leader, are different. Most significantly, they are synthesized under different growth conditions. DsrA is made when cells are growing at low temperature and provides the cell with adequate RpoS to deal with low-temperature growth (Repoila F and Gottesman S. *J Bacteriol* 183: 4012-23,

The eukaryotic microRNAs and RNAi have recently gained a great deal of attention. In prokaryotes such as Escherichia coli, *up to 100 such ncRNAs have been identified and found to regulate a variety of processes.*

2001). Sequences within the *dsrA* core promoter directly confer temperature sensitivity on this promoter. In contrast, RprA is made in response to cell surface stress; its promoter carries a binding site for an activator called RcsB (Majdalani N et al. *Mol Microbiol* 46: 813-26, 2002). Furthermore, each small ncRNA can have multiple targets. Thus, at low temperature DsrA upregulates RpoS but also downregulates another bacterial regulatory protein, called HNS (Sledjeski D and Gottesman S. *Proc Natl Acad Sci U S A* 92: 2003-7, 1995; Lease RA and Belfort M. *Proc Natl Acad Sci U S A* 97: 9919-24, 2000).

Yet other small ncRNAs negatively regulate targets. The small ncRNA RyhB is made when cells are depleted of iron, a situation most pathogens face when infecting their hosts. This small ncRNA also pairs with its target mRNAs, in this case causing their rapid degradation. The targets all encode genes that store and use iron, but they are not essential. Thus, by downregulating these genes, iron becomes available for more essential functions (Massé E and Gottesman S. *Proc Natl Acad Sci U S A* 99: 4620-5, 2002).

The use of small ncRNA regulators is not limited to *E. coli*. Many of the small ncRNAs and their targets are conserved in pathogenic species of *Klebsiella, Salmonella, Vibrio*, and *Yersinia*, and related small ncRNAs have recently been found in *Pseudomonas*. Even in *Staphylococcus aureus*, translation of the α toxin is regulated by a hairpin loop disrupted by a small RNA called RNAIII (Morfeldt E et al. *EMBO J* 14: 4569-77, 1995).

The discovery of small ncRNAs that are made in response to environmental signals and can positively or negatively regulate groups of genes greatly expands the regulatory repertoire of all cells. Synthesis of these small ncRNAs in response to different environmental cues provides the cell with the flexibility to coordinate and integrate multiple signal transduction pathways at the relatively cheap cost of making an RNA of 100 nucleotides, as opposed to protein regulators. In bacteria, these small ncRNAs may play important roles in pathogenesis and could provide novel targets for antibiotics.

- **Nadim Majdalani, PhD**
  Staff Scientist
- **Susan Gottesman, PhD**
  Principal Investigator
  Laboratory of Molecular Biology
  NCI-Bethesda, Bldg. 37/Rm. 5132
  Tel: 301-496-3524
  Fax: 301-496-3875
  susang@helix.nih.gov

SCIENTIFIC ADVISORY COMMITTEE

If you have scientific news of interest to the CCR research community, please contact one of the scientific advisors (below) responsible for your area of research.

Biotechnology Resources

David J. Goldstein, PhD
dg187w@nih.gov
Tel: 301-496-4347

David J. Munroe, PhD
dm368n@nih.gov
Tel: 301-846-1697

Carcinogenesis, Cancer and Cell Biology, Tumor Biology

Joseph A. DiPaolo, PhD
jd81a@nih.gov
Tel: 301-496-6441

Stuart H. Yuspa, MD
sy12j@nih.gov
Tel: 301-496-2162

Clinical Research

Frank M. Balis, MD
fb2y@nih.gov
Tel: 301-496-0085

Caryn Steakley, RN, MSW
cs397r@nih.gov
Tel: 301-435-3685

Immunology

Jonathan D. Ashwell, MD
ja9s@nih.gov
Tel: 301-496-4931

Jay A. Berzofsky, MD, PhD
jb4q@nih.gov
Tel: 301-496-6874

Molecular Biology

Ira O. Daar, PhD
id13e@nih.gov
Tel: 301-846-1667

Carl Wu, PhD
cw1m@nih.gov
Tel: 301-496-3029

Structural Biology, Chemistry

Christopher J. Michedja, PhD
cm304t@nih.gov
Tel: 301-846-1216

Sriram Subramaniam, PhD
ss512h@nih.gov
Tel: 301-594-2062

Translational Research

Anita B. Roberts, PhD
ar40e@nih.gov
Tel: 301-496-6108

Elise C. Kohn, MD
ek1b@nih.gov
Tel: 301-402-2726

Virology

Vinay K. Pathak, PhD
vp63m@nih.gov
Tel: 301-846-1710

John T. Schiller, PhD
js153g@nih.gov
Tel: 301-496-6539

The Receptor Interacting Protein Plays an Essential Role in DNA Damage-induced NF-κB Activation

Hur GM, Lewis J, Yang Q, Lin Y, Nakano H, Nedospasov S, and Liu ZG. The death domain kinase RIP has an essential role in DNA damage-induced NF-κB activation. *Genes Dev* 17: 873-82, 2003.

Cells are often under genotoxic stress, both endogenously (e.g., reactive oxygen species) and exogenously (e.g., ultraviolet radiation, ionizing radiation, and DNA-damaging chemicals). The cellular responses to genotoxic stress include damage sensing, activation of different signaling pathways, and biological consequences such as cell cycle arrest and apoptosis. Tremendous work has been done to investigate the mechanisms that control the cellular responses to genotoxic stress.

Transcription factors p53, activating protein 1 (AP-1), and nuclear factor κB (NF-κB) have been suggested to play critical roles in mediating cellular responses to genotoxic stesses. These transcription factors elicit various biological responses through induction of their target genes' expression.

Because activation of AP-1 and NF-κB can have anti-apoptotic or pro-apoptotic effects, the engagement of these two pathways may be key cellular responses that modulate the outcome of cells exposed to radiation and genotoxic drugs. For instance, the ataxia-telangiectasia mutation gene (ATM) has been reported to play an essential role in genotoxic drug-induced activation of NF-κB, which has a known role in chemoresistance. Although ATM may be critical for sensing DNA damage and subsequently triggering cytoplasmic signaling cascades that lead to NF-κB activation, how the signal is transduced from nucleus to cytoplasm and how the cytoplasmic signaling cascades are initiated are largely unknown.

In most types of cells, inactive NF-κB is sequestered in the cytoplasm through its interaction with the inhibitory proteins known as IκBs. In response to various stimuli, such as the pro-inflammatory cytokines tumor necrosis factor (TNF) and interleukin 1 (IL-1), IκBs are phosphorylated by their kinase, IKK, and are rapidly degraded by the proteasome after polyubiquitination. Degradation of IκBs leads to the release of NF-κB and allows its translocation into the nucleus and the subsequent activation of its targets' genes.

For TNF-induced IKK and NF-κB activation, two TNF effector molecules are essential, the death domain kinase receptor interacting protein (RIP) and TNF receptor-associated factor 2 (TRAF2). In response to TNF treatment, IKK is recruited to the TNF receptor complex through TRAF2, and its activation requires RIP. RIP interacts with the IKK complex through the regulatory subunit IKK-γ; apparently this interaction also requires TRAF2.

Ionizing radiation and short-wavelength ultraviolet radiation activate NF-κB through distinct pathways. Ultraviolet radiation activates NF-κB through an IKK-independent pathway. For ionizing radiation and genotoxic drugs, DNA damage has been proposed to trigger a cytoplasmic signaling cascade, including IKK activation, to mediate NF-κB activation. The signaling components upstream of IKK have not been identified, however. Previous studies had implied that ultraviolet radiation and genotoxic drugs may activate NF-κB through cell membrane receptors (e.g., TNF and IL-1 receptors). Thus, it is important to determine whether RIP is involved in NF-κB activation by ultraviolet radiation or genotoxic drugs.

We found that RIP is essential for NF-κB activation by genotoxic drugs and ionizing radiation but not by ultraviolet radiation. In wild-type mouse embryonic fibroblasts, DNA damage by agents such as Adriamycin, camptothecin, and ionizing radiation induced NF-κB activation. In contrast, NF-κB activation could not be detected in $RIP^{-/-}$ fibroblasts treated with these agents. NF-κB activation by IL-1β and lipopolysaccharide treatment was normal in $RIP^{-/-}$ fibroblasts compared with wild-type fibroblasts.

To test whether TNF receptor or other TNF signaling effector molecules (e.g., TRAF2, FADD, and TRAF5) are involved in DNA damage-induced NF-κB activation, we examined NF-κB activation in $TNF\text{-}R1^{-/-}$, $TRAF2^{-/-}$, $TRAF5^{-/-}$, and $FADD^{-/-}$ fibroblasts and found that NF-κB was activated normally in those cells. Therefore, the involvement of RIP in DNA damage-induced NF-κB activation is independent of TNF signaling.

Because IKK forms a complex with RIP after DNA damage, RIP may activate IKK through the same mechanism in response to both TNF and DNA damage. However, how RIP senses the signal triggered by DNA damage is still unclear. Since RIP is not a nucleocytoplasmic shuttling protein, one possibility is that, in response to DNA damage, some nuclear proteins are exported into the cytoplasm and interact with RIP to form signaling complexes with IKK. Nevertheless, our work suggests a novel mechanism for the initiation of the cytoplasmic signaling to activate NF-κB in response to DNA damage.

■ **Zheng-Gang Liu, PhD**
Principal Investigator
Cell and Cancer Biology Branch
NCI-Bethesda, Bldg. 10/Rm. 6N105
Tel: 301-435-6351
Fax: 301-402-1997
zgliu@box-z.nih.gov

A New Paradigm for Multidisciplinary Collaboration: The Molecular Targets Development Program

Translation of basic discoveries into effective molecularly targeted therapeutic agents within the CCR has, in the past, faced a major rate-limiting step. Our ability to identify promising molecular targets relevant to cancer, AIDS, and other human diseases has outstripped our capacity to validate such targets for therapeutic intervention and to develop high-throughput assays for identifying novel compounds aimed at perturbing their biological activity. To close this gap and leverage in-house expertise in exploiting high-throughput screening strategies to identify new bioprobes and inhibitory molecules, the CCR and the Molecular Targets Faculty established the Molecular Targets Development Program (MTDP).

Under the directorship of James B. McMahon, PhD, the MTDP has become the centerpiece of CCR's high-throughput screening efforts. The program's goals are to facilitate the discovery of compounds that may serve as inhibitors or bioprobes for functional genomics, proteomics, and molecular target validation research and to identify potential lead compounds for targeted drug development. Compounds of interest to MTDP investigators include not only classical small "drug-like" molecules but also peptides and other bioactive chemical classes. A primary strength of the research group is its long-standing expertise in the chemistry of natural products—the class of compounds that has yielded or inspired the design of the majority of new chemical entities introduced as drugs since 1980 (Newman DJ et al. *J Nat Prod* 66: 1022-37, 2003).

To reduce the conceptual and temporal lag between discovery and development, the MTDP laboratory is organized to facilitate the iterative flow of information between biologists and chemists. The lab comprises four major research focus areas: 1) target validation, bioassay development, and screening de-replication, 2) natural products chemistry, 3) protein chemistry, and 4) molecular biology and phage display.

Researchers in the first focus area develop, adapt, evaluate, and apply contemporary screening assays and emerging technologies for target validation and lead discovery. They use their expertise for bioassay-guided lead isolation of pure compounds contained in diverse libraries derived from natural, synthetic, and biosynthetic sources. Models can encompass receptor binding; immunoassay; enzyme assay; protein-protein, -nucleic acid, and -carbohydrate interactions; gene expression; and functional assays. The lab's capacity for high-throughput screening has recently increased substantially with the acquisition of the Discovery-1 imaging system, which can conduct cell-based assays in microtiter plates with densities of 384 wells per plate.

Our ability to identify promising molecular targets relevant to cancer, AIDS, and other human diseases has outstripped our capacity to validate such targets for therapeutic intervention and to develop high-throughput assays for identifying novel compounds aimed at perturbing their biological activity.

MTDP staff scientists with expertise in natural products chemistry engage in bioassay-guided fractionation, isolation, purification, and structural elucidation of novel leads derived from natural products. They possess extensive knowledge of the NCI Natural Products Repository, which contains nearly 150,000 unique samples, and implement new chemical and spectroscopic techniques to facilitate structural analysis. In addition to having access to the extraordinary chemical diversity of the repository, the MTDP accesses or maintains several other chemical libraries, including the structural and mechanistic diversity libraries of NCI's Developmental Therapeutics Program, a 6,000-member pure natural products set, and a collection of synthetic compounds purchased from Chembridge, Inc. Libraries are also being acquired from collaborators at Boston University and the University of Pittsburgh.

In the area of protein chemistry, MTDP investigators assist in bioassay development; conduct the fractionation, purification, and structural analysis of novel anti-tumor proteins and peptides derived from the program's chemical diversity libraries; and investigate mechanisms of action. The group also quantitates the thermodynamic interactions between proteins and their biologically relevant ligands and can structurally modify proteins to optimize interactions with molecular targets.

MTDP's molecular biologists have emphasized constructing a series of in-house phage-display peptide libraries (greater than 10^{10} diversity) to expand biology-driven chemical diversity in the CCR and to apply the technology directly to molecularly targeted cancer research. Phage display can be used to discover novel ligands for specific molecular

Continued on page 8…

…continued from page 7

targets and in many other applications. The MTDP has recently acquired a set of phage-display human antibody libraries from the Medical Research Council, UK. The molecular biology section also assists in bioassay development and in protein and peptide characterization.

As this abbreviated description suggests, MTDP research focus groups engage in highly interactive and frequently complementary activities, thereby encouraging multidisciplinary approaches to target validation and lead compound development. Over the past 2 years, MTDP multidisciplinary teams have contributed to the development of several molecularly targeted agents, ranging from inhibitors of cyclin-dependent kinases, the Akt pathway, SMAD signal transduction, and ABC multidrug transporters to inhibitors of V-ATPases, ubiquitin ligase, and HIV-1 Rnase H. An exciting collaboration with CCR's Keith Robertson, PhD, was recently established to develop a high-throughput screen for inhibitors of DNA methyl transferase 1. This research into how factors involved in regulating epigenetic gene silencing during normal development go awry in cancer may inspire the development of novel therapeutic agents that restore normal methylation patterns and re-establish growth control in cancer cells.

All principal investigators are encouraged to collaborate in research with MTDP multidisciplinary project teams. Initial contact is frequently informal and is followed up by a formal presentation of the investigator's research to the MTDP Steering Committee. For a full description of the application process, please refer to the MTDP website at http://ccr.cancer.gov/labs/lab.asp?labid=112.

■ **J.Carl Barrett, PhD**

CCR Frontiers in Science—Staff

Center for Cancer Research
J. Carl Barrett, PhD, *Director*
Kathryn Zoon, PhD, *Principal Deputy Director*
Frank M. Balis, MD, *Clinical Director*
L. Michelle Bennett, PhD, *Associate Director for Science*

Deputy Directors
C. Norman Coleman, MD
Lee J. Helman, MD
Douglas R. Lowy, MD
Robert H. Wiltrout, PhD
Stuart H. Yuspa, MD

Editorial Staff
Tracy Thompson, *Editor-in-Chief*
Sue Fox, BA/BSW, *Senior Editor*
Lizzie Hess, ELS(D), *Managing Editor**
Ave Cline, *Editor*
Kay Fleming, PhD, *Scientific Writer*
Kathryn Ellis, *Intern*
Rob Wald, *Publications Manager**
Michael Fleishman, *Graphic Artist**

*Palladian Partners, Inc.

■ ADMINISTRATIVE LINKS

Changes to the Telephone System

As of December 1, 2003, NIH transitioned to a new General Services Administration telecommunications contract. This change affects employees because 5-digit dialing is now discontinued and has been replaced with 10-digit dialing. Please correct all pre-programmed dialing on telephone sets, facsimile equipment, and computer systems. To learn more go to http://www.cit.nih.gov/dnst/DNSTweb/telephone.html.

Acceptance of Premium Class Tickets from Sponsors

The policy concerning acceptance of premium class tickets from sponsors was altered in June 2003. A premium class ticket from a sponsor can be accepted; however, two Federal Travel Regulation (FTR) provisions have to be met along with one of the four HHS conditions. The first FTR provision is that the non-Federal source makes full payment in "advance of the travel" (i.e., an In Kind ticket or In Cash reimbursement is provided to NIH before the trip). The second FTR provision is that the accommodations furnished are comparable in value to those offered to, or purchased by, other similarly situated persons attending the meeting or similar function.

To learn more about the HHS provisions please see the November 2003 NCI Administrative Newsletter at http://camp.nci.nih.gov/admin/news/admin/200311/. The NCI Travel Coordinator, Anne Rogerson, can address any questions at rogersoa@mail.nih.gov.

A-76 Update

In the fall of 2003 NIH's Most Efficient Organization won the bid for the Extramural Administrative Support Services, and NIH also announced winning the second of the two large cost comparisons in Real Property Management (RPM). The new RPM organization is scheduled to take effect on March 31, 2004. With the new RPM organization, NIH plans to save more than 200 positions in this area. NCI does not have any employees involved in this reorganization; however, the winning bid will rely on centralization of services and may result in a new process for receiving services.

In November 2003 the discussion of Subject Matter Experts that will be assigned to the pre-planning committees for Information Technology, Committee Management, Visual Information/Medical Arts, and Veterinary Support positions scheduled for competition in 2004 was forwarded to the NIH Deputy director for Management and the NIH A-76 Steering Committee. To learn more visit OMA's A-76 website at http://camp.nci.nih.gov/admin/oem/mab/A76online.htm or NIH's A-76 website at http://a-76.nih.gov. Questions can also be submitted through OMA's website to the A-76 program manager.