Department of Biomedical Genetics and University of Rochester Stem Cell and Regenerative Medicine Institute, University of Rochester Medical Center, Rochester, NY 14642, USA
[b] Department of Pathology, Ohio State University Wexner Medical Center, 410W 10th Avenue, E403 Doan Hall, Columbus, OH 43210-1240, USA
[c] University of Michigan Tech Transfer, 1600 Huron Pkwy, 2nd Floor, Building 520, Ann Arbor, MI 48109-2590, USA
[d] Department of Radiation Oncology, University of Maryland School of Medicine,10 South Pine Street, MSTF Room 600, Baltimore, MD 21201, USA
[e] Department of Neurology, Massachusetts General Hospital, Harvard Medical School, 55 Fruit Street, Yawkey 9E, Boston, MA 02114, USA
[f] Harvard Medical School, Department of Cell Biology 240 Longwood Avenue Building C1, Room 513B Boston, MA 02115, USA
[g] University of Colorado School of Medicine, Division of Hematology, 12700 E. 19th Avenue, Campus Box F754-AMCA, Aurora, CO 80045, USA

a r t i c l e i n f o

Article history:
Received 23 May 2014
Received in revised form
29 October 2014
Accepted 30 October 2014
Available online 4 December 2014

Keywords:
Redox
Stem cell
Progenitor cell
Oligodendrocyte/type-2 astrocyte progenitor cell
c-Cbl
Redox/Fyn/c-Cbl pathway
Glioblastoma
Basal-like breast cancer
Tumor initiating cell
Tamoxifen

a b s t r a c t

This review discusses a unique discovery path starting with novel findings on redox regulation of precursor cell and signaling pathway function and identification of a new mechanism by which relatively small changes in redox status can control entire signaling networks that regulate self-renewal, differentiation, and survival. The pathway central to this work, the redox/Fyn/c-Cbl (RFC) pathway, converts small increases in oxidative status to pan-activation of the c-Cbl ubiquitin ligase, which controls multiple receptors and other proteins of central importance in precursor cell and cancer cell function. Integration of work on the RFC pathway with attempts to understand how treatment with systemic chemotherapy causes neurological problems led to the discovery that

Contents

that progenitors from the cortex, which myelinates late and over a long time period, undergo extensive self-renewal in vitro under basal division conditions and are relatively resistant to inducers of oligodendrocyte generation

ubiquitinated by c-Cbl, it also appears that activation of c-Cbl is modulated by Fyn rather than by an activated RTK.

multiforme (GBM), multiple myeloma, and lymphoma) and which also can be used as part of a preparatory (conditioning) regimen prior to bone marrow transplantation. BCNU treatment has been associated with signi cant changes in mental status and with white matter degeneration [108,109]. Cisplatin is a DNA cross-linking agent used in treatment of various kinds of cancers, including sarcomas, some carcinomas, lymphomas, and germ cell tumors. At high doses, it has been associated with leukoencephalopathy and destruction of CNS white matter [110]. The antimetabolite cytarabine is a nucleoside analog used mainly in treatment of white blood cell tumors, such as acute myeloid leukemia, acute lymphocytic leukemia, and lymphomas. Application of cytarabine has also been associated with acute encephalopathy, confusion, memory loss, and white matter changes [110,111]. 5-Fluorouracil (5-FU) is a pyrimidine analog that inhibits thymidylate synthase and is used alone or as an important component of adjuvant

cells) in which vulnerability was comparable to that observed for primary neural progenitor cells, with most such cell lines being more resistant to these agents than the normal cells. This outcome was seen despite the fact that the cancer cell lines we studied often were chosen because of their previous use in studies on the response to the drugs studied.

In vitro analyses of puri ed cell populations were highly predictive of effects seen following systemic treatment with any of the chemotherapeutic agents in vivo, in terms of both the cellular targets and the ability of exposure to these agents to cause cell death and also to suppress precursor cell division. In particular, in vitro studies showed that doses of BCNU, cisplatin, and cytarabine that did not kill normal precursor cells were nonetheless suf cient to suppress their division

BCNU and TMX are prooxidants—and TMX is potentially a very useful prooxidant for treatment of a wide range of cancers

Multiple standard anticancer agents appear to make cells more oxidized. It was thus a natural question for us to ask whether the effects of these agents on O-2A/OPCs might be mediated through activation of the RFC pathway.

The agents we chose for our rst studies were BCNU and TMX.

We found that Cool-1/βpix—but not Cdc42—is a critical inhibitor of c-Cbl function in GBM cells (summarized in Fig. 6). In GBM

reactivation. Knockdown of Cool-1/

studies on BLBC cells, we found identical results on 7 independent BLBC cell lines representing Basal B triple-negative cells, Basal A cells, and Basal A cells with Her2 ampli cation [241]. In our studies on GBMs, ve independent GBM cell lines all yielded identical results, as did examination of biopsies from ve different patients [227]. In addition, analysis of ve c-Cbl targets in GBM samples (via the Cancer Genome Atlas) showed increased levels of at least two c-Cbl target proteins in 73% of cases and of at least three c-Cbl targets in 43% of cases. A more focused examination of tumors with increased EGFR protein expression showed increased expression of Cool-1/βpix mRNA in 65% of samples, and in 52% of all GBM samples in the database. With the caveat that these databases provide no information on isoform changes and alterations in Cool-1/β

knockdown cells to TMX caused a further decrease in EGFR levels [241]. In addition, it also was recently reported that transglutaminase-2 also can inhibit c-Cbl function in some GBM cell lines [361].

There also is a growing list of proteins known to regulate the activity of Cool-1/βpix and Cdc42, and each of these is of potential interest as a means of controlling c-Cbl activity. For example, inhibition of a particularly central activator of Cool-1/βpix might produce outcomes that were similar to knockdown of Cool-1/βpix itself. In addition, it has been reported that MEK inhibition also decreases Cool-1/β-pix phosphorylation [365], providing another possible means of regulating this inhibitor of c-Cbl.

The ways in which proteins that might regulate c-Cbl interact

of cancer cells is a puzzling one, and understanding how this can be accomplished is likely to provide insights important for developing better anticancer therapies. How a MEK1/2 inhibitor might have such different effects on normal cells and transformed cells is not yet known. It is curious, however, that there are previous observations indicating that MEK inhibition can also decrease Cool-1/β-pix phosphorylation [365]. It will be of great interest to determine if the MEK inhibitors we discovered through investigations on protective strategies have the unexpected property of also being able—at least in some cancers—of restoring normal c-Cbl function. If so, their value in cancer treatment may be considerable, but also would bene

analyses of the role of redox state in cell signaling do not argue against the importance of more standard approaches to analysis of signaling pathway function. Instead, it is the integration of all of these various approaches together that is likely to offer the greatest power going forward. Indeed, we suspect that one of the greatest opportunities going forward will be to combine our work with studies on genetic networks that also are shared by multiple cancers and that arise in speci c response to the action of cooperating oncogenes. The network of cooperation response genes (CRGs) was rst identi ed in studies by Hartmut Land and colleagues on cooperation between dominant-negative p53 and constitutively active Ras^{V12} (Ras) mutants in a model of colon cell transformation [382]. Systematic functional analysis demonstrated that CRGs are highly enriched for regulators of malignancy, and resetting expression of individual CRGs to normal cell levels suppressed growth of both experimental tumors and naturally occurring human tumors [382,383]. Restoring normal expression of even a single CRG was, in most cases, suf cient to reduce tumor growth, and similar effectiveness was seen in both experimentally induced cancers and xenograft tumor formation by human cancer cells [382–384].

Regulation of cell transformation by CRGs appears to be a general principle, as CRGs that regulate transformation of colon cells play a role in multiple epithelial tumor types, and particularly in cancers (such as BLBCs) in which p53 and Ras pathway mutations are often found. Although there are distinct CRG sets

[27]

[141] Han, R.; Yang, Y. M.; Dietrich, J.; Luebke, A.; Mayer-Proschel, M.; Noble, M. Systemic 5- uorouracil treatment causes a syndrome of delayed myelin destruction in the central nervous system. J Biol 7:12; 2008.
[142] Dessi, F.; Pollard, H.; Moreau, J.; Ben-Ari, Y.; Charriaut-Marlangue, C. Cytosine arabinoside induces apoptosis in cerebellar neurons in culture. J. Neurochem. 64:1980–1987; 1995.
[143] Geller, H. M.; Cheng, K. -Y.; Goldsmith, N. K.; Alejandro, A. A.; Zhang, A. -L.; Morris, E. J.; Grandison, L. Oxidative stress mediates neuronal DNA damage and apoptosis in response to cytosine arabinoside. J. Neurochem. 78:265–275; 2001.
[144] Martin, D. P.; Wallace, T. L.; Johnson, E. M. J. Cytosine arabinoside kills postmitotic neurons in a fashion resembling trophic factor deprivation: evidence that a deoxycytidine-dependent process may be required for nerve growth factor signal transduction. J. Neurosci. 10:184–193; 1990.
[145] Sanz-Rodriguez, C.; Boix, J.; Comella, J. X. Cytosine arabinoside is neurotoxic to chick embryo spinal cord motoneurons in culture. Neurosci. Lett. 223:141–144; 1997.
[146] Tomkins, C. E.; Edwards, S. N.; Tolkovsky, A. M. Apoptosis is induced in post-mitotic rat sympathetic neurons by arabinosides and topoisomerase II inhibitors in the presence of NGF. J. Cell Sci. 107:1499–1507; 1994.
[147] Wick, A.; Wick, W.; Hirrlinger, J.; Gerhardt, E.; Dringen, R.; Dichgans, J.; Weller, M.; Schulz, J. B. Chemotherapy-induced cell death in primary cerebellar granule neurons but not in astrocytes: in vitro paradigm of differential neurotoxicity. J. Neurochem 91:1067–1074; 2004.
[148] Cho, K. H.; Choi, S. M.; Kim, B. C.; Lee, S. H.; Park, M. S.; Kim, M. K.; Kim, J. K. 5- uorouracil-induced oligodendrocyte death and inhibitory effect of cyclo-heximide, Trolox, and Z-VAD-FMK in murine cortical culture. Cancer 100:1484–1490; 2004.
[149] Yamada, M.; Nakagawa, H.; Fukushima, M.; Shimizu, K.; Hayakawa, T.; Ikenaka, K. In vitro study on intrathecal use of 5- uoro-2'-deoxyuridine (FdUrd) for meningeal dissemination of malignant brain tumors. J. Neuroon-col. 37:115–121; 1998.
[150] Rowitch, D. H.; Lu, Q. R.; Kessaris, N.; Richardson, W. D. An 'oligarchy' rules neural development. Trends Neurosci 25:417–422; 2002.
[151] Ross, S. E.; Greenberg, M. E.; Stiles, C. D. Basic helix-loop-helix factors in cortical development. Neuron 39:13–25; 2003.
[152] Ligon, K. L.; Fancy, S. P.; Franklin, R. J.; Rowitch, D. H. Olig gene function in CNS development and disease. Glia 54:1–10; 2006.
[153] Li, H.; Richardson, W. D. The evolution of Olig genes and their roles in myelination. Neuron glia biology 4:129–135; 2008.
[154] Mitew, S.; Hay, C. M.; Peckham, H.; Xiao, J.; Koenning, M.; Emery, B. Mechanisms regulating the development of oligodendrocytes and central nervous system myelin. Neuroscience ; 2013.
[155] Brezden, C. B.; Phillips, K. A.; Abdolell, M.; Bunston, T.; Tannock, I. F. Cognitive function in breast cancer patients receiving adjuvant chemotherapy. Journal of clinical oncology: official journal of the American Society of Clinical Oncology 18:2695–2701; 2000.
[156] Haykin, M. E.; Gorman, M.; van Hoff, J.; Fulbright, R. K.; Baehring, J. M. Diffusion-weighted MRI correlates of subacute methotrexate-related neuro-toxicity. J. Neurooncol. 76:153–157; 2006.
[157]

proliferation and induces memory de cits in rats. Behavioural brain research 201:279–284; 2009.

factor in the cycling of Cdc42 to regulate insulin secretion. American journal of physiology. Endocrinology and metabolism 301:E1072–1080; 2011.
[240] Kim, S.; Kim, D.; Lee, S. H.; Park, H.; Kim, H.; Park, D. Molecular cloning of neuronally expressed mouse betaPix isoforms. Biochem. Biophys. Res. Commun. 272:721–725; 2000.
[241] Chen, H. -Y.; Yang, Y. M.; Stevens, B.; Noble, M. Inhibition of redox/Fyn/c-Cbl pathway function by Cdc42 controls tumor initiation capacity and tamoxifen sensitivity in basal-like breast cancer cells. EMBO Mol. Med 5:723–736; 2013.
[242] Pichot, C. S.; Arvanitis, C.; Hartig, S. M.; Jensen, S. A.; Bechill, J.; Marzouk, S.; Yu, J.; Frost, J. A.; Corey, S. J. Cdc42-interacting protein 4 promotes breast cancer cell invasion and formation of invadopodia through activation of N-WASp. Cancer Res. 70:8347–8356; 2010.
[243] Dombrosky-Ferlan, P.; Grishin, A.; Botelho, R. J.; Sampson, M.; Wang, L.; Rudert, W. A.; Grinstein, S.; Corey, S. J. Felic (CIP4b), a novel binding partner with the Src kinase Lyn and Cdc42, localizes to the phagocytic cup. Blood 101:2804–2809; 2003.
[244] Surviladze, Z.; Waller, A.; Strouse, J. J.; Bologa, C.; Ursu, O.; Salas, V.; Parkinson, J. F.; Phillips, G. K.; Romero, E.; Wandinger-Ness, A.; Sklar, L. A.; Schroeder, C.; Simpson, D.; Nöth, J.; Wang, J.; Golden, J.; Aubé, J. A Potent and Selective Inhibitor of Cdc42 GTPase. SourceProbe Reports from the NIH Molecular Libraries Program [Internet]. Bethesda (MD): National Center for Biotechnology Information (US) 2010; Feb 27; 2010.
[245] Rizzo, P.; Miao, H.; D'Souza, G.; Osipo, C.; Song, L. L.; Yun, J.; Zhao, H.; Mascarenhas, J.; Wyatt, D.; Antico, G.; Hao, L.; Yao, K.; Rajan, P.; Hicks, C.; Siziopikou, K.; Selvaggi, S.; Bashir, A.; Bhandari, D.; Marchese, A.; Lendahl, U.; Qin, J. Z.; Tonetti, D. A.; Albain, K.; Nickoloff, B. J.; Miele, L. Cross-talk between notch and the estrogen receptor in breast cancer suggests novel therapeutic approaches. Cancer Res. 68:5226–5235; 2008.
[246] Baumann, M.; Krause, M.; Hill, R. Exploring the role of cancer stem cells in radioresistance. Nature reviews. Cancer 8:545–554; 2008.
[247] Visvader, J. E.; Lindeman, G. J. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nature reviews. Cancer 8:755–768; 2008.
[248] O'Brien, C. A.; Kreso, A.; Dick, J. E. Cancer stem cells in solid tumors: an overview. Seminars in radiation oncology 19:71–77; 2009.
[249] Diehn, M.; Cho, R. W.; Clarke, M. F. Therapeutic implications of the cancer stem cell hypothesis. Seminars in radiation oncology 19:78–86; 2009.
[250] Scopelliti, A.; Cammareri, P.; Catalano, V.; Saladino, V.; Todaro, M.; Stassi, G. Therapeutic implications of Cancer Initiating Cells. Expert opinion on biological therapy 9:1005–1016; 2009.
[251] Rosen, J. M.; Jordan, C. T. The increasing complexity of the cancer stem cell paradigm. Science 324:1670–1673; 2009.
[252] Gupta, P. B.; Chaffer, C. L.; Weinberg, R. A. Cancer stem cells: mirage or reality? Nat Med 15:1010–1012; 2009.
[253] Frank, N. Y.; Schatton, T.; Frank, M. H. The therapeutic promise of the cancer stem cell concept. The Journal of clinical investigation 120:41–50; 2010.
[254] Wan, F.; Zhang, S.; Xie, R.; Gao, B.; Campos, B.; Herold-Mende, C.; Lei, T. The utility and limitations of neurosphere assay, CD133 immunophenotyping and side population assay in glioma stem cell research. Brain Pathol 20:877–889; 2010.
[255] Greaves, M. Cancer stem cells: back to Darwin? Seminars in cancer biology 20:65–70; 2010.
[256] Cheng, L.; Bao, S.; Rich, J. N. Potential therapeutic implications of cancer stem cells in glioblastoma. Biochemical pharmacology 80:654–665; 2010.
[257] Stratford, A. L.; Reipas, K.; Maxwell, C.; Dunn, S. E. Targeting tumour-initiating cells to improve the cure rates for triple-negative breast cancer. Expert reviews in molecular medicine 12:e22; 2010.
[258] Garvalov, B. K.; Acker, T. Cancer stem cells: a new framework for the design of tumor therapies. J Mol Med (Berl) 89:95–107; 2011.
[259] D'Angelo, R. C.; Wicha, M. S. Stem cells in normal development and cancer. Progress in molecular biology and translational science 95:113–158; 2010.
[260] Blanpain, C.; Mohrin, M.; Sotiropoulou, P. A.; Passegue, E. DNA-damage response in tissue-speci c and cancer stem cells. Cell Stem Cell 8:16–29; 2011.
[261] Soltanian, S.; Matin, M. M. Cancer stem cells and cancer therapy. Tumour biology: the journal of the International Society for Oncodevelopmental Biology and Medicine 32:425–440; 2011.
[262] Clevers, H. The cancer stem cell: premises, promises and challenges. Nat Med 17:313–319; 2011.
[263] Rasheed, Z. A.; Kowalski, J.; Smith, B. D.; Matsui, W. Concise review: Emerging concepts in clinical targeting of cancer stem cells. Stem Cells 29:883–887; 2011.
[264] Frame, F. M.; Maitland, N. J. Cancer stem cells, models of study and implications of therapy resistance mechanisms. Advances in experimental medicine and biology 720:105–118; 2011.
[265] Pietras, A. Cancer stem cells in tumor heterogeneity. Advances in cancer research 112:255–281; 2011.
[266] Ghiaur, G.; Gerber, J.; Jones, R. J. Concise review: Cancer stem cells and minimal residual disease. Stem Cells 30:89–93; 2012.
[267] Li, Y.; Laterra, J. Cancer stem cells: distinct entities or dynamically regulated phenotypes? Cancer research 72:576–580; 2012.
[268] Tang, D. G. Understanding cancer stem cell heterogeneity and plasticity. Cell research 22:457–472; 2012.
[269] McCubrey, J. A.; Steelman, L. S.; Abrams, S. L.; Misaghian, N.; Chappell, W. H.; Basecke, J.; Nicoletti, F.; Libra, M.; Ligresti, G.; Stivala, F.; Maksimovic-Ivanic, D.; Mijatovic, S.; Montalto, G.; Cervello, M.; Laidler, P.; Bonati, A.; Evangelisti, C.; Cocco, A. M.; Martelli, A. M. Targeting the cancer initiating cell: the ultimate target for cancer therapy. Current pharmaceutical design 18:1784–1795; 2012.
[270] Magee, J. A.; Piskounova, E.; Morrison, S. J. Cancer stem cells: impact, heterogeneity, and uncertainty. Cancer Cell 21:283–296; 2012.
[271]

[296] Tatarek, J.; Cullion, K.; Ashworth, T.; Gerstein, R.; Aster, J. C.; Kelliher, M. A. Notch1 inhibition targets the leukemia-initiating cells in a Tal1/Lmo2 mouse model of T-ALL. Blood 118:1579–1590; 2011.
[297] Wang, J.; Sullenger, B. A.; Rich, J. N. Notch signaling in cancer stem cells. Advances in experimental medicine and biology 727:174–185; 2012.
[298] Ponnurangam, S.; Mammen, J. M.; Ramalingam, S.; He, Z.; Zhang, Y.; Umar, S.; Subramaniam, D.; Anant, S. Honokiol in combination with radiation targets notch signaling to inhibit colon cancer stem cells. Molecular cancer therapeutics 11:963–

[350] Engel, R. H.; Evens, A. M. Oxidative stress and apoptosis: a new treatment