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Chemical genetic approaches to probing cell death

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Chemical genetics has arisen as a tool for the discovery of pathways and proteins in mammalian systems. This approach, comprising small-molecule screening combined with biochemical and genomic target identification methods, enables one to assess which proteins are involved in regulating a particular phenotype. Applied to cell death, this strategy can reveal novel targets and pathways regulating the demise of mammalian cells. Numerous diseases have been linked to the loss of regulation of cell death. Defining the mechanisms governing cell death in these diseases might lead to the discovery of therapeutic agents and targets and provide a richer understanding of the mortality of living systems. Recent advances include the discovery of novel small molecules regulating cell death pathways — necrostatin and erastin — as well as the elucidation of the mechanism of death induced in cancer cells by the cytotoxic agent Apratoxin A.

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Introduction

Regulation of cell death is essential for normal development and maintenance of tissue homeostasis in metazoans [1]. Metazoan cells die through several different stereotypical modes, most notably through apoptosis or necrosis. When such cells lose responsiveness to death-inducing stimuli, aberrant cell survival can result in tumor formation [2]. By mapping the pathways that are inactivated in tumor cells, we might discover ways to overcome their resistance to death stimuli and reengage the cell death machinery. Targeting some of these pathways might enable tumor-selective lethality.

Conversely, proper maintenance of the nervous system requires that neurons produced during development continue to survive throughout adulthood. Unfortunately, neurodegenerative diseases lead to premature neuronal death and consequent loss of proper brain function. Diseases such as Huntington's, Parkinson's, Alzheimer's, amyotrophic lateral sclerosis and spinal muscular atrophy have all been linked to premature and selective neuronal death [3]. Defining the mechanism of neuronal death in each case might lead to the development of therapeutic agents that promote neuronal survival. Thus, knowledge of cell death pathways might facilitate the discovery of treatments for two classes of intractable diseases: cancer and neurodegeneration.

The earliest insights into the molecular basis of cell death come from genetic studies in the nematode *Caenorhabditis elegans* [4]. Wild-type nematodes are consistently made up of 959 cells [5] that can be easily visualized in their transparent bodies [6]. These features make *C. elegans* suitable for observing alterations in organismal cell number. Through a series of single gene mutations, genes that enhance or inhibit cell death were found; similar work has been performed in *Drosophila* [7•]. Orthologous pathways operate in mammals; however, there might be other mammalian-specific pathways to identify. Unfortunately, large-scale genetic screens are difficult to undertake in mammalian systems.

Chemical genetics has arisen as a tool for the discovery of pathways and proteins in mammalian systems, including human cells [8,9••]. Using small molecules as molecular probes, one can search, for example, for mechanisms specifically regulating the death of mammalian cells. Strategies for target identification with such compounds involve genetic and genomic screens [10••,11•,12•,13], and affinity purification of target proteins [14]. These approaches can sometimes enable identification of the proteins responsible for small-molecule-induced effects.

Thus far, the most studied pathways governing cellular destruction are those of apoptosis, which occur mostly either through extrinsic death receptor pathways or the intrinsic mitochondrial pathway [15,16]. Whereas variations have been identified within this framework [17], apoptotic pathways are predominantly carried out by a family of cysteine proteases that cleave at aspartic acid residues, known as caspases [18]. Less well defined is necrotic death, which had been thought to occur in an uncontrolled fashion as a response to tissue injury. More recent reports, however, suggest that necrotic death also follows a defined cellular program, and is, in fact, genetically regulated [19••].

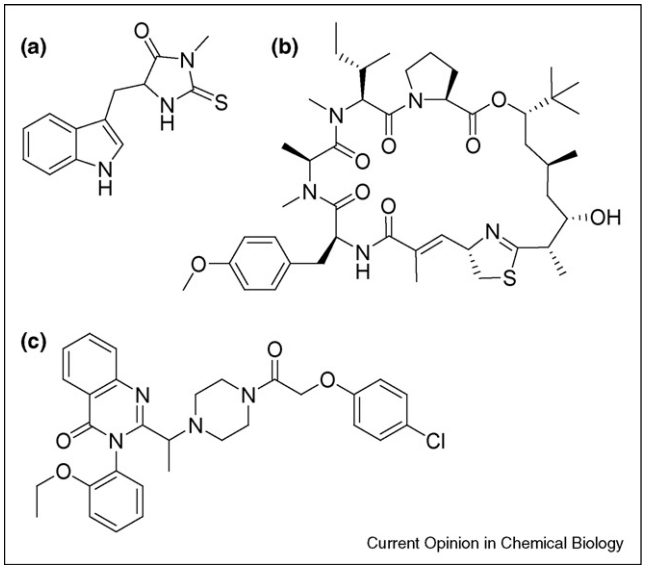
Here, we discuss recent advances in the understanding of cell death pathways made possible by the use of small

molecules, and the potential for future discoveries in this field.

Role of histone deacetylase inhibitors in regulating cell death

Histone deacetylase (HDAC) inhibitors are a class of small molecules that has been implicated in regulating both cell death and cell survival. Such compounds reverse the transcriptional repression of HDACs, which can lead to cell cycle arrest, or cell death [20]. Therefore, several HDAC inhibitors have been used to target cancer cells. Notable examples of potent HDAC inhibitors include trapoxins A and B [21], trichostatin A [22] and suberoylanilide hydroxamic acid (SAHA) [23]. All have proven to be effective cytotoxic agents in cancer cells *in vitro* with an acceptable therapeutic index; SAHA was recently approved for use in the USA by the FDA [24]. Interestingly, owing to diverse cellular responses to transcriptional regulation, other HDAC inhibitors such as valproic acid and phenylbutyrate are also undergoing clinical trials to prevent motor neuron death in SMA patients [25].

Figure 1



Chemical structures of **(a)** necrostatin-1 **(b)** aprotoxin A and **(c)** erastin.

Discovery and inhibition of a novel death pathway, necroptosis

Degterev *et al.* [26••] identified a compound that can be used to define how necrotic death commences. Because of the traditional view that necrosis does not proceed through an orderly progression of cellular events, most therapies to protect cells from death target apoptotic pathways. However, Degterev *et al.* described a necrosis-like death pathway, which they term 'necroptosis', and have identified a chemical inhibitor of this pathway. Necroptosis, as it has been defined, occurs when death is induced by a death receptor ligand, for example, tumor necrosis factor α (TNFα), in the presence of broad-spectrum caspase inhibition, such as with z-VAD-fmk. Under these conditions, cells proceed to die, displaying characteristic features of necrosis. This type of death is thought to occur when an apoptotic stimulus occurs in an environment that is stressed in a way that prevents the execution of apoptosis, as in ischemic brain injury, where energy levels are low because of oxygen deprivation [27].

Of 15 000 compounds screened against human monocytes provoked to undergo necroptosis, one compound, necrostatin-1 (Nec-1, Figure 1a), was identified, which inhibits necroptosis. Several assays of necrotic death were performed, including tests of ATP levels, mitochondrial permeability, plasma membrane permeability, proliferation and morphological analyses. No features of necrosis remained detectable in the presence of Nec-1. This compound also prevented autophagy that occurs following necroptosis but had no effect on autophagy when induced independently of necroptosis, suggesting Nec-1 acts fairly early in the sequence of 'necroptotic' events.

A structure–activity relationship analysis of 71 Nec-1 analogs showed a 'spiky' structure–activity landscape: few positions were found at which modifications could be made, but one more potent analog was found: 7-chloro-Nec-1 [28•]. Although these findings would suggest that Nec-1 does bind to one or more specific targets, the identity of such targets has not been defined. However, some insight into the targeted pathway comes from the finding that Nec-1 was able to prevent death induced by dimerization of RIP kinases. These death-receptor-interacting proteins are activated downstream of death receptor signaling, and their activity leads to the execution of necroptotic events in the absence of Nec-1.

Because necroptosis is implicated in ischemic brain injury, the ability of Nec-1 to reduce infarct size in mice was tested following middle cerebral artery occlusion, a stroke-emulating condition. Nec-1 provided protection against ischemia, as demonstrated by a reduction in infarct size when administered either pre- or up to 6 h post-occlusion. This protection further supports the occurrence, as well as the persistence, of necroptosis following ischemic brain injury. Whereas apoptosis immediately follows the induced injury, necroptosis is ongoing; coadministration of z-VAD-fmk and Nec-1 produced an additive effect in reducing infarct size. Therefore, the discovery of Nec-1 not only suggests the existence of a specific necrosis-like pathway leading to cell death (i.e. necroptosis), but also has physiological relevance and the potential for advancing therapeutic development for stroke treatment.

Mode of death induced by cytotoxic agent apratoxin A

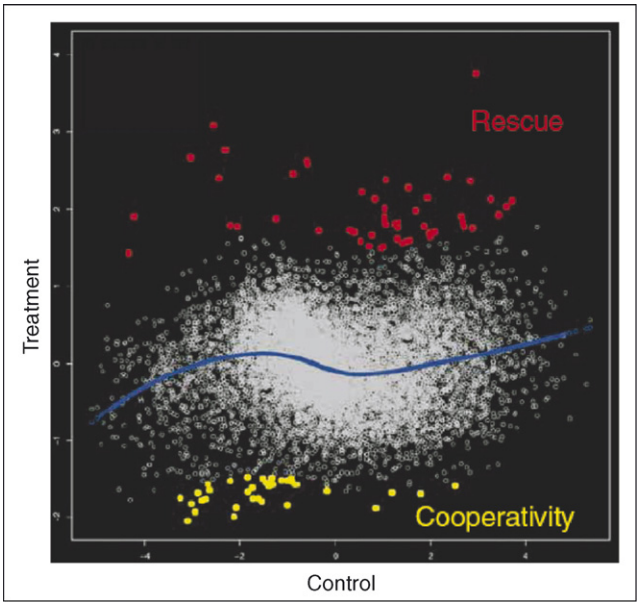
Developing treatments for cancer involves the flip side to the problem of understanding cell death: how do we prevent unwanted cells from surviving and proliferating? Luesch *et al.* [29••] have described a compound, apratoxin A (AprA, Figure 1b), which they have used to probe tumor cell death. As with many natural products isolated from cyanobacteria [30–33], AprA acts as a cytotoxic agent and has been tested previously for antitumor activity [34]. Although it proved effective in eliminating cancer cells, the pathway by which AprA acts was elusive. Luesch *et al.* [29••] have used mRNA expression profiling to determine that AprA induces disruption of cell cycle progression, leading to G1 arrest; this result was confirmed by DNA content analysis [29••]. Once treated, cells containing diploid DNA accumulate and fail to proceed to S phase. Additionally, higher doses of AprA result in apoptosis as demonstrated by the presence of sub-G1 DNA content, indicating fragmentation, and increased detection of DEVDase activity, which is used as a proxy for increased caspase 3 and caspase 7 activity [29••].

To understand more specifically how AprA-treated cells were dying, Luesch *et al.* [29••] performed a genome-wide overexpression screen of 27 000 genes. Because the activity of a compound is sensitive to the expression level of its target protein, for compounds that act via a loss of function, knocking down their target makes cells more sensitive to the activity of the compound, whereas overexpressing the target makes cells resistant. The opposite is true for compounds that act via a gain of function: knocking down their target makes cells resistant, whereas overexpressing their target increases the cell's sensitivity. Therefore, an overexpression screen for resistance should find targets of compounds that act via a loss of function (i.e. those that inhibit their target [Figure 2]).

An overexpression screen identified several fibroblast growth factor (FGF) receptors (FGFRs) that prevent G1 arrest in response to AprA, two of which (FGFR1 and FGFR3) are preferentially expressed at high levels in tumor cell lines resistant to AprA. Furthermore, the phosphorylation of a downstream target of FGFR signaling, STAT3, is inhibited in the presence of AprA, and a constitutively active STAT3 mutant protects cells from death in the presence of AprA. Conversely, small interfering RNA (siRNA) targeted against signal transducer and activators of transcription 3 (STAT3) directly decreases viability of U20S cells. Although these data are not sufficient to define a direct target of AprA activity, they indicate that some component of the FGFR/STAT3 pathway is probably involved [29••].

FGF signaling and STAT3 activation have been previously implicated in tumor invasion and metastasis [35•,36]. Therefore, Luesch *et al.* [29••] performed an *in vitro* angiogenesis assay to test the role of AprA on the ability of cultured human umbilical cord endothelial cells (HUVECs) to form tube-like structures. The presence of AprA reduced capillary tube sprouting to a similar extent as, although more potently than, a known FGFR inhibitor that is also anti-angiogenic. Additionally, an *in vivo* zebrafish assay showed reduced expression of the FGF target gene *mkp3* in AprA-treated animals. Reduction of *mkp3* expression leads to defects in pectoral fin development, a typical consequence of downregulated FGF signaling [37], as well as defects in tail development that are specific to AprA-mediated FGF inhibition. Therefore, AprA must have additional effects on zebrafish development that are distinct from those of other FGFR inhibitors. Thus, further investigation into the action of AprA might lead to the development of drugs that exploit the FGF/STAT3 signaling pathway to prevent tumor invasion.

Figure 2



Statistical analysis of an overexpression screen. A two-dimensional fitting model, LOWESS (locally weighted regression and smoothing scatterplots), was used to analyze an overexpression screen of 27 000 cDNAs in 50nM AprA-treated (treatment) and 25% ethanol-treated (control) U20S cells. This analysis revealed cDNAs that prevent AprA-induced toxicity (rescue, red) and cDNAs that enhance toxicity of AprA (cooperativity, yellow). The blue line represents the fitted curve from LOWESS analysis. Reproduced, with permission, from [29••].

Erastin selectively targets H-Ras-overexpressing tumor cells

Another lethal compound, erastin (Figure 1c), was isolated in a screen of 23 550 compounds for genotype-selective lethality [38]. By measuring shifts in IC_{50} values in engineered tumorigenic cell lines containing defined

genetic changes, Dolma *et al.* [38] isolated several known genotype-specific lethal compounds. In the screen, viability of cells was determined by calcein AM, a fluorescent dye that is selectively retained in live cells. By performing such a genotype-selective screen, it was possible to identify compounds that selectively inhibit only cells that display oncogenic phenotypes of interest, leaving non-tumorogenic cells unharmed. One novel compound, erastin, was selective for tumor cells harboring oncogenic H-Ras.

Erastin-induced death in Ras-overexpressing cells was found to be non-apoptotic — no fragmentation or margination of chromatin was visible. Cells did not show DNA laddering, which occurs when DNA is cleaved at regular intervals and serves as a hallmark of apoptotis. Annexin V staining was absent, and caspase 3, a final executioner of apoptosis, remained in its inactivate, procaspase form. Additionally, the pan-caspase inhibitor BOC-D-fmk did not block death induced by erastin. Therefore, in addition to identifying a Ras-selective lethal compound, it appears that the discovery of erastin has uncovered a novel mechanism for killing cells. Further work to identify the mode of action for erastin is ongoing.

Conclusions

Thus far, studies using chemical genetics have advanced our knowledge of several death pathways in numerous cell types. This approach could also be applied to other enigmatic cell death processes. A death pathway of interest that has been difficult to unravel is the one that occurs in olfactory receptor neurons (ORNs), the sensory neurons of the olfactory system. Residing in the olfactory epithelium lining the olfactory cavity within the nose, these cells, which serve to detect odors in the environment [39], are prone to death and represent one of the few neuronal populations, along with granule cells in the olfactory bulb [40] and the dentate gyrus of the hippocampus [41], that die and are continually replaced throughout adulthood [42]. Owing to this rare characteristic, along with their position in a stratified epithelium containing only one neuronal type [43], ORNs stand out as a model cell type for the study of neurodegeneration. As yet, the mechanism of their death is unknown, but cannot be blocked by typical cell death inhibitors (Gangadhar NM, Stockwell BR and Firestein SJ, unpublished). The use of chemical genetics might be useful to shed light on the mechanism of ORN death as well as other elusive cell death pathways.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Schafer ZT, Kornbluth S: **The apoptosome: physiological, developmental, and pathological modes of regulation**. *Dev Cell* 2006, **10**:549-561.

2. Malaguarnera L: **Implications of apoptosis regulators in tumorigenesis**. *Cancer Metastasis Rev* 2004, **23**:367-387.

3. Ekshyyan O, Aw TY: **Apoptosis: a key in neurodegenerative disorders**. *Curr Neurovasc Res* 2004, **1**:355-371.

4. Metzstein MM, Stanfield GM, Horvitz HR: **Genetics of programmed cell death in *C. elegans*: past, present and future**. *Trends Genet* 1998, **14**:410-416.

5. Sulston JE, Horvitz HR: **Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans***. *Dev Biol* 1977, **56**:110-156.

6. Ward S, Thomson N, White JG, Brenner S: **Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans***. *J Comp Neurol* 1975, **160**:313-337.

7. • Hay BA, Huh JR, Guo M: **The genetics of cell death: approaches, insights and opportunities in *Drosophila***. *Nat Rev Genet* 2004, **5**:911-922.
This review describes similarities in the cell death machinery of flies, worms and mammals. The authors discuss advantages of using *Drosophila* to study cell death pathways, including ease of targeting specific genes, number of available fly lines containing single-gene mutations, the ability to drive tissue-specific gene expression, spatial and temporal control over RNAi-mediated knockdown, and genetic screening strategies to identify genes of interest.

8. Huang Z: **The chemical biology of apoptosis. Exploring protein–protein interactions and the life and death of cells with small molecules**. *Chem Biol* 2002, **9**:1059-1072.

9. •• Stockwell BR: **Exploring biology with small organic molecules**. *Nature* 2004, **432**:846-854.
This review discusses the potential to explore biological activity with small organic molecules. Parallels between molecular genetics and chemical genetics are drawn; challenges to chemical genetics are presented and addressed. Finally, the author discusses strategies for screening in high-throughput and target identification.

10. •• Moffat J, Grueneberg DA, Yang X, Kim SY, Kloepfer AM, Hinkle G, Piqani B, Eisenhaure TM, Luo B, Grenier JK *et al.*: **A lentiviral RNAi library for human and mouse genes applied to an arrayed viral high-content screen**. *Cell* 2006, **124**:1283-1298.
This group has developed lentiviral short hairpin RNA (shRNA) libraries targeting the human and murine genomes. To demonstrate the power and applicability of this resource, they use the library to screen for genes regulating mitotic progression in human cancer cells.

11. • Pritsker M, Ford NR, Jenq HT, Lemischka IR: **Genomewide gain-of-function genetic screen identifies functionally active genes in mouse embryonic stem cells**. *Proc Natl Acad Sci USA* 2006, **103**:6946-6951.
These researchers performed a genetic overexpression screen using a genome-wide cDNA library. With the use of microarray detection methods and high-throughput functional analyses, they identified genes that regulate differentiation in mouse embryonic stem (ES) cells. The methods they discuss can be applied to screening for various phenotypes in ES cells and other cell lines of interest.

12. • Beissbarth T, Speed TP: **GOstat: find statistically overrepresented Gene Ontologies within a group of genes**. *Bioinformatics* 2004, **20**:1464-1465.
This paper describes software that has been developed to analyze data accumulated from microarray experiments, or other experiments that produce long lists of genes. The program, GOstat, takes information from the Gene Ontology (GO) database, which contains functional annotations of all genes, to identify which annotations are statistically overrepresented within a given list of genes.

13. Root DE, Hacohen N, Hahn WC, Lander ES, Sabatini DM: **Genome-scale loss-of-function screening with a lentiviral RNAi library**. *Nat Methods* 2006, **3**:715-719.

14. Mondal K, Gupta MN: **The affinity concept in bioseparation: evolving paradigms and expanding range of applications**. *Biomol Eng* 2006, **23**:59-76.

15. Wajant H: **The Fas signaling pathway: more than a paradigm**. *Science* 2002, **296**:1635-1636.

16. Mattson MP, Kroemer G: **Mitochondria in cell death: novel targets for neuroprotection and cardioprotection**. *Trends Mol Med* 2003, **9**:196-205.

17. Yuan J, Lipinski M, Degterev A: **Diversity in the mechanisms of neuronal cell death**. *Neuron* 2003, **40**:401-413.

18. Boyce M, Degterev A, Yuan J: **Caspases: an ancient cellular sword of Damocles**. *Cell Death Differ* 2004, **11**:29-37.

19. •• Nicotera P, Melino G: **Regulation of the apoptosis-necrosis switch**. *Oncogene* 2004, **23**:2757-2765.
This review examines the factors that determine which type of cell death will progress in various physiological conditions, and how various death pathways intertwine. The authors suggest that ATP levels and nitric oxide are both key regulators in determining the mechanism and morphology of death.

20. Marks PA, Richon VM, Breslow R, Rifkind RA: **Histone deacetylase inhibitors as new cancer drugs**. *Curr Opin Oncol* 2001, **13**:477-483.

21. Itazaki H, Nagashima K, Sugita K, Yoshida H, Kawamura Y, Yasuda Y, Matsumoto K, Ishii K, Uotani N, Nakai H *et al.*: **Isolation and structural elucidation of new cyclotetrapeptides, trapoxins A and B, having detransformation activities as antitumor agents**. *J Antibiot (Tokyo)* 1990, **43**:1524-1532.

22. Furumai R, Komatsu Y, Nishino N, Khochbin S, Yoshida M, Horinouchi S: **Potent histone deacetylase inhibitors built from trichostatin A and cyclic tetrapeptide antibiotics including trapoxin**. *Proc Natl Acad Sci USA* 2001, **98**:87-92.

23. Spiller SE, Ravanpay AC, Hahn AW, Olson JM: **Suberoylanilide hydroxamic acid is effective in preclinical studies of medulloblastoma**. *J Neurooncol* 2006, **79**:259-270.

24. Duvic M, Talpur R, Ni X, Zhang C, Hazarika P, Kelly C, Chiao JH, Reilly JF, Ricker JL, Richon VN *et al.*: **Phase II trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL)**. *Blood* 2006, in press.

25. Wirth B, Brichta L, Schrank B, Lochmuller H, Blick S, Baasner A, Heller R: **Mildly affected patients with spinal muscular atrophy are partially protected by an increased SMN2 copy number**. *Hum Genet* 2006, **119**:422-428.

26. •• Degterev A, Huang Z, Boyce M, Li Y, Jagtap P, Mizushima N, Cuny GD, Mitchison TJ, Moskowitz MA, Yuan J: **Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury**. *Nat Chem Biol* 2005, **1**:112-119.
This paper describes a novel pathway leading to cell death, in which an apoptotic stimuli leads to death that displays characteristics of necrosis (termed 'necroptosis'). The authors perform a screen to find a small molecule inhibitor of this pathway, and use this inhibitor to demonstrate the physiological relevance of this type of death in an induced stroke model in mice.

27. Lou M, Chen Y, Ding M, Eschenfelder CC, Deuschl G: **Involvement of the mitochondrial ATP-sensitive potassium channel in the neuroprotective effect of hyperbaric oxygenation after cerebral ischemia**. *Brain Res Bull* 2006, **69**:109-116.

28. • Teng X, Degterev A, Jagtap P, Xing X, Choi S, Denu R, Yuan J, Cuny GD: **Structure–activity relationship study of novel necroptosis inhibitors**. *Bioorg Med Chem Lett* 2005, **15**:5039-5044.
A structure–activity relationship of 71 analogs of novel necroptosis inhibitor, necrostatin-1 (Nec-1) shows that most positions are highly sensitive to substitutions; however, variations at the indole 7 position increased necroptosis inhibitory activity.

29. •• Luesch H, Chanda SK, Raya RM, DeJesus PD, Orth AP, Walker JR, Izpisua Belmonte JC, Schultz PG: **A functional genomics approach to the mode of action of apratoxin A**. *Nat Chem Biol* 2006, **2**:158-167.
This study describes the mode of action of cytotoxic agent aprotoxin A (AprA), as revealed by a genome-wide overexpression screen for resistance. Overexpression of several FGFRs confers resistance to AprA toxicity; AprA inhibits the phosphorylation of the FGFR target STAT3, resulting in AprA-induced death.

30. Williams PG, Yoshida WY, Moore RE, Paul VJ: **Tasiamide, a cytotoxic peptide from the marine cyanobacterium *Symploca* sp**. *J Nat Prod* 2002, **65**:1336-1339.

31. Williams PG, Yoshida WY, Moore RE, Paul VJ: **Tasipeptins A and B, new cytotoxic depsipeptides from the marine cyanobacterium *Symploca* sp**. *J Nat Prod* 2003, **66**:620-624.

32. Williams PG, Yoshida WY, Moore RE, Paul VJ: **Micromide and guamamide: cytotoxic alkaloids from a species of the marine cyanobacterium *Symploca***. *J Nat Prod* 2004, **67**:49-53.

33. Williams PG, Yoshida WY, Quon MK, Moore RE, Paul VJ: **Ulongapeptin, a cytotoxic cyclic depsipeptide from a Palauan marine cyanobacterium *Lyngbya* sp**. *J Nat Prod* 2003, **66**:651-654.

34. Luesch H, Yoshida WY, Moore RE, Paul VJ, Corbett TH: **Total structure determination of apratoxin A, a potent novel cytotoxin from the marine cyanobacterium *Lyngbya majuscula***. *J Am Chem Soc* 2001, **123**:5418-5423.

35. • Xie TX, Huang FJ, Aldape KD, Kang SH, Liu M, Gershenwald JE, Xie K, Sawaya R, Huang S: **Activation of Stat3 in human melanoma promotes brain metastasis**. *Cancer Res* 2006, **66**:3188-3196.
Increases in STAT3 activity were discovered in human brain metastatic melanoma cells. Furthermore, transfection with constitutively active or dominant negative STAT3, respectively, enhances or suppresses brain metastasis, and alterations in STAT3 activity affect angiogenesis.

36. Xie TX, Wei D, Liu M, Gao AC, Ali-Osman F, Sawaya R, Huang S: **Stat3 activation regulates the expression of matrix metalloproteinase-2 and tumor invasion and metastasis**. *Oncogene* 2004, **23**:3550-3560.

37. Kawakami Y, Esteban CR, Matsui T, Rodriguez-Leon J, Kato S, Belmonte JC: **Sp8 and Sp9, two closely related buttonhead-like transcription factors, regulate Fgf8 expression and limb outgrowth in vertebrate embryos**. *Development* 2004, **131**:4763-4774.

38. Dolma S, Lessnick SL, Hahn WC, Stockwell BR: **Identification of genotype-selective antitumor agents using synthetic lethal chemical screening in engineered human tumor cells**. *Cancer Cell* 2003, **3**:285-296.

39. Buck L, Axel R: **A novel multigene family may encode odorant receptors: a molecular basis for odor recognition**. *Cell* 1991, **65**:175-187.

40. Carleton A, Petreanu LT, Lansford R, Alvarez-Buylla A, Lledo PM: **Becoming a new neuron in the adult olfactory bulb**. *Nat Neurosci* 2003, **6**:507-518.

41. Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH: **Neurogenesis in the adult human hippocampus**. *Nat Med* 1998, **4**:1313-1317.

42. Calof AL, Rim PC, Askins KJ, Mumm JS, Gordon MK, Iannuzzelli P, Shou J: **Factors regulating neurogenesis and programmed cell death in mouse olfactory epithelium**. *Ann N Y Acad Sci* 1998, **855**:226-229.

43. Kawauchi S, Beites CL, Crocker CE, Wu HH, Bonnin A, Murray R, Calof AL: **Molecular signals regulating proliferation of stem and progenitor cells in mouse olfactory epithelium**. *Dev Neurosci* 2004, **26**:166-180.