Singapore signalling: the 2012 hedgehog pathway cocktail

James Briscoe & Rajat Rohatgi

The ‘Hedgehog Signalling in Development Evolution and Disease’ conference took place in Biopolis, Singapore, in March 2012. Organized by Phil Ingham with help from Xinhua Lin, Mary-Ann Price and Fred de Sauvage, it brought leading researchers together to discuss the latest findings and exchange ideas on every aspect of hedgehog signalling.

Singapore, with its glittering hi-tech skyscrapers sitting alongside nineteenth century colonial buildings, provided a perfect backdrop for the meeting. The scientific sessions were punctuated by dinners of chilli crab and grilled skate, an homage to Singapore’s transcendental cuisine. In the year that the first drug to target the hedgehog pathway reached the market, introductory talks by David Lane, Chief Scientist of the Agency for Science, Technology and Research (A*Star, Singapore), and Matthew Scott (Stanford U., USA) reflected on the 32-year journey from discovery of the pathway in Drosophila to an effective cancer drug. Over the past decades, the study of hedgehog has taken many unexpected turns and led to the discovery of new regulatory principles in remarkably diverse areas of cell and developmental biology. Importantly, in an age of increasing emphasis on translational research, the story of hedgehog provides a resounding endorsement for basic research and its potential to directly impact human health.

Production and spread

Several speakers tackled how Hh proteins are secreted and moved through tissues. One distinct feature of Hh is its proteolytic processing and modification by the addition of two lipids—cholesterol and palmitate—during its production to yield the dually lipidated secreted form Hh-N. How does such a hydrophobic molecule escape the plasma membrane to spread from cell to cell? Phil Beachy (Stanford U., USA) presented evidence that the secreted molecule Scube2—identified through the ‘you’ mutant in zebrafish1—can promote the solubilization and release of lipidated, processed Sonic Hedgehog (Shh)-N from cultured cells or from isolated detergent-resistant lipid rafts [2]. The efficacy of Scube2 is enhanced by the palmitoyl adduct on Hh-N and by the co-expression of Dispatched (Disp), a transporter-like protein previously implicated in Hh secretion. In this regard, Adrian Salic (Harvard U., USA) showed that the cholesterol adduct of Hh-N can be cross-linked to Disp. These data suggest the interesting idea that proteins involved in Hh secretion might specifically recognize the lipid modifications on Hh-N.

Pascal Therond (Institute of Biology Valrose, France) and Isabel Guerrero (Centro de Biología Molecular ‘Severo Ochoa’, U. Madrid, Spain) described characterization of Hh secreted from cultured cells. Immunoelectron microscopy revealed that a significant proportion of secreted Hh associates with microvesicles 50–200nm in diameter. Therond’s lab found that these particles contain ESCRT proteins and Guerrero’s lab found them to contain TSG101 and Alix/Alp1. The presence of these markers suggested the idea that these microvesicles are exosomes derived from the intraluminal vesicles of multivesicular bodies (MVBs). Therond speculated that Hh at the cell surface is internalized by a Disp-dependent mechanism and targeted to secretory MVBs. Marta Swierczynska (MPI-Dresden, Germany) described two distinct forms of Shh secreted from human cells ectopically expressing the protein. Some Shh-N associated with different lipoprotein classes, including low-density lipoprotein (LDL) particles. A second species was monomeric and lacked its cholesterol moiety. Consistent with LDLs being important in the spread and activity of Shh, Anabel Christ (Max-Delbrück Center for Molecular Medicine, Germany) showed that LRP2, a LDL receptor family member, promotes signalling in vivo by binding to Shh and controlling the internalization and transporting of Patched 1 (Ptc1)–Shh complexes [3].

...iHog and Boi, co-receptors for Hh, promote the formation of cytonemes and the localization of Hh to cytonemes

Continuing the theme of Hh movement, Xinhua Lin (Chinese Academy of Sciences, Beijing, China) and Christian Siebold (U. Oxford, UK) emphasized the importance of extracellular proteins containing heparin sulphate chains. A heparin-sulphate-interacting Cardin–Weintraub domain has been reported in Shh, but Siebold noted that this domain is not highly conserved across species and its elimination has only moderate effects on the signalling capacity

Three of the main themes to emerge from the meeting concerned new understanding of how hedgehog (Hh) ligands are produced and spread through tissues, how cells transduce Hh signals, and the roles of Hh signalling in normal tissue and disease states.
of Shh. Taking a crystallographic approach, Siebold reported the structure of Shh-N in a complex with heparin sulphate. In addition to the Cardin–Weintraub domain, heparin sulphate bound to a positively charged surface of conserved amino acids in Shh located adjacent to the putative Ptch1-interacting region. The crystal structure suggested that a heparin-sulphate chain could form the template for the formation of a linear array of Hh-N. Siebold speculated that such an arrangement might be responsible for the subsequent clustering of receptors such as Cdo and Ptch1.

Among the long list of ciliary proteins [...] it is important to distinguish [...] those that indirectly affect [Hh] signalling by compromising cilia structure or function

Finally, Guerrero reported new evidence supporting an important role for ‘cytonemes’ in the spread of Hh through tissue. These actin-based, filopodia-like extensions have been implicated in morphogen movement for more than a decade but details have remained elusive [4]. Guerrero presented beautiful images of cytonemes extending from basal–lateral membranes of wing disc cells, and found that iHog and Boi, co-receptors for Hh, promote the formation of cytonemes and the localization of Hh to cytonemes. She speculated that exosomes containing Hh are transported along cytonemes through tissue. Elucidation of the function, molecular composition and regulation of cytonemes will clearly be a main focus of research in the future.

Signalling

Much attention has naturally focused on the reception and transduction of Hh signals. Several proteins have been implicated in binding to Hh to initiate intracellular signal transduction. Ben Allen (U. Michigan, USA) discussed the role of three of these—Gas1, Cdo and Boc—which are required for Hh signalling in many tissues, in which they seem to act as co-receptors that bind to Hh, and function with Ptch1, to transduce the signal. For example, embryos lacking Gas1 and Cdo have holoprosencephaly, a characteristic of reduced Shh signalling. Strikingly, however, holoprosencephaly is partly rescued in Gas1/Boc double mutants indicating that Boc might also have an inhibitory role, at least in some situations. On the basis of Allen’s and Guerrero’s presentations, these proteins clearly have functions beyond acting as simple co-receptors for Hh.

A central player in Hh transduction is Ptch1, the 12-pass transmembrane protein that represses the activity of a 7-pass transmembrane protein, Smoothened (Smo). The binding of Hh ligands to Ptch1 relieves inhibition of Smo. The molecular mechanism by which Ptch1 regulates Smo remains mysterious and we did not get many insights into this important question at this meeting. What is clear, however, is that the signal transduction mechanism from Smo to downstream effectors requires primary cilia in vertebrates. Several speakers added fresh detail to this feature of the pathway. Jonathan Eggenschwiler (Princeton U., USA) provided evidence that the cilia-associated cell-cycle-related kinase CCRK is required for Shh signalling in a similar manner to its binding partner Bromi [5]. He showed that mouse embryonic fibroblasts lacking CCRK have a defect in the kinetics of Gli2 recruitment to the tips of cilia on Shh stimulation. Rajat Rohatgi (Stanford U., USA) detailed the function of EVC2, a gene originally identified in patients who suffer from a ciliopathy characterized by impaired Hh signalling in skeletal tissues. The absence of Evc2 did not seem to affect cilia integrity. However, Hh signalling promoted the binding of Evc2 to Smo at a previously uncharacterized region of the cilia just distal to the transition zone. Among the long list of ciliary
proteins implicated in Hh signalling over the past decade, it is important to distinguish between those that are dedicated to Hh signalling as opposed to those that indirectly affect signalling by compromising cilia structure or function.

...new and unexpected functions of Hh signalling continue to surface [...] Hh ligands secreted from damaged or inflamed tissues can shape the adaptive immune response

Downstream from Smo, PKA and Sufu are the two main negative regulators of the Gli family of transcription factors. How Smo activation overcomes PKA and Sufu, probably at primary cilia, is another important unanswered question in signalling. Kazushi Aoto (Stowers Institute for Medical Research, USA) presented evidence that AKAP11, a member of a scaffolding protein family that regulates PKA localization in subcellular compartments, binds to both Ptch1 and Smo, and RNA interference of AKAP11 results in ectopic signalling in the chick spinal cord. This might provide clues as to how PKA activity or localization is regulated by Hh signalling. Rune Toftgard (Karolinska Institutet, Sweden) presented the crystal structure of full-length SuFu and identified a flexible regulatory subdomain encompassing an 80-amino-acid segment disordered in the crystals, which is required for Hh signalling to overcome the negative effect of SuFu, but surprisingly is not required for the ability of SuFu to inhibit Gli proteins. The flexible subdomain might be the crucial sequence element that connects SuFu to upstream signalling.

Downstream from Smo, Hh signalling ultimately regulates the activity of zinc finger-containing transcription factors of the Gli/Ci family. These are bifunctional transcriptional regulators, inhibiting transcription in the absence of signalling and activating transcription in response to Hh signalling. Jiang Wu (U. Texas Southwestern, USA) provided evidence that the ability of the GliIs to both activate and repress transcription depends on an interaction with Brg1, a component of the Swi–Snf complex. In addition, Scott Atwood (Stanford U., USA) presented data suggesting that atypical protein kinase C (aPKC) directly phosphorylates the zinc-finger motif of Gli1 and enhances Gli1 DNA binding, a mechanism that might have therapeutic relevance in basal cell carcinomas (see below).

Tissue development
One of the best-described biological functions of Shh signalling is its role as a graded signal that directs pattern formation in the vertebrate neural tube. How a signalling gradient is converted into different gene expression programmes is a fascinating question, the answer to which probably reveals fundamental principles of transcriptional control. Work from James Briscoe (MRC National Institute for Medical Research, UK) and Andrew McMahon (Harvard U., USA) demonstrated the importance of probing the dynamics of the gene regulatory network through developmental time. By using a combination of experimental work and computational modelling, Briscoe emphasized that the network of transcription factors downstream from Shh signalling is crucial for the graded response, by directly contributing to the differential spatial and temporal control of gene expression patterns [6]. Cliff Tabin (Harvard U., USA) added an interesting evolutionary dimension to this problem by asking how such patterning mechanisms can accommodate large size differences, such as those seen between the neural tubes of chick and zebrafish embryos.

...Ptch2 contributes to inhibition of Shh signalling in the skin and raises the question of why an inhibitory activity of Ptch2 on Shh signalling is not evident in other tissues

Tabin also described a new role for Shh signalling in a long-studied Hh-responsive tissue: the limb. Removal of Smo from the muscle progenitors migrating into the limb resulted in a 30% decrease in slow twitch muscle fibres, and muscle progenitors failed to migrate all the way to the autopod—the feet. A transcriptome screen identified the Rho guanine nucleotide exchange factors Net1 and Dock9 as Hh target genes necessary for this migration. Consistent with this, reactivation of Rhoa in muscle progenitors that lacked Shh signalling rescued the migration of progenitors into the autopod.

In addition to the established roles, new and unexpected functions of Hh signalling continue to surface, especially in adult organ homeostasis and injury responses. Tessa Crompton (U. College London, UK) described how Hh ligands secreted from damaged or inflamed tissues can shape the adaptive immune response by skewing CD4 T-cell differentiation towards a T₂ lineage, exacerbating the severity of T₂-driven pathology such as that seen in allergic asthma. Michael Galko (U. Texas M. D. Anderson Cancer Center, USA) presented evidence that Hh signalling modulates pain perception in both Drosophila and mammals [7]. Such talks emphasized that Hh-targeted therapeutics might find broad and unexpected medical uses.

Cancer connections
A customary topic in every Hh meeting is the role of this signalling pathway in tumour formation and growth. Several cases of cancer that produce Shh have been identified. Beachy described one example—bladder cancer. Basal cells underlying the bladder epithelium express Shh, and genetic lineage analysis in the mouse revealed that these are the stem cells from which the epithelium is regenerated after injury [8]. Moreover, it is these same cells that are the origin of tumours when the epithelium is exposed to chronic mutagenesis; ablation of Shh-expressing cells before mutagenesis prevented tumour formation. Alex Swarbrick (Garvan Institute, Australia) observed the expression of Shh in tumour cells from the basal-like subtype of breast cancer. In particular, aggressive basal breast cancers were enriched for ligand production, and an Shh antibody decreased tumour volume and metastatic spread in a mouse model of the disease. These two studies highlight the importance of understanding the mechanism of Shh expression in tumour cells and whether it is a viable therapeutic target.

In contrast to ligand-producing tumours, the skin cancer basal cell carcinoma and the cerebellum cancer medulloblastoma, are caused by the cell-autonomous mutational activation of the pathway. Frederic Charron (U. Montreal, Canada) showed that the expression of Boc is upregulated in early medulloblastomas. The genetic removal of Boc in Ptch1 heterozygous mice did not change the frequency of pre-neoplastic lesions; however, it caused a marked reduction in the size of tumours and rescued 65%...
Although Hh inhibitors are clearly useful in tumours […] caused by the cell-autonomous activation of the pathway—their role in tumours displaying ligand overexpression remains uncertain of the lethality in these mice. He proposed that Boc in important in the progression but not the initiation of medulloblastoma. In the case of basal cell carcinoma, Brandon Wainwright (Institute for Molecular Bioscience, U. Queensland, Australia) provided evidence that the level of Shh signalling influenced the extent and nature of the lesion. Deletion of Ptch1 from skin progenitor cells resulted in ectopic Shh signalling and hyperplasia but no conspicuous basal cell carcinoma lesions. By contrast, removal of both Ptch1 and Ptch2—a close paralogue of Ptch1—completely blocked skin differentiation without resulting in basal cell carcinoma. This indicates that Ptch2 contributes to inhibition of Shh signalling in the skin and raises the question of why an inhibitory activity of Ptch2 on Shh signalling is not evident in other tissues.

Several pharmaceutical companies are pursuing drug discovery programmes targeting the Hh pathway. Fred de Sauvage (Genentech, USA), who led the translation of the first approved Hh cancer drug Vismodegib, summarized the clinical results and the travails that culminated in FDA approval for this agent in inoperable basal cell carcinoma. Although Hh inhibitors are clearly useful in tumours, such as basal cell carcinoma and medulloblastoma—caused by the cell-autonomous activation of the pathway—their role in tumours displaying ligand overexpression remains uncertain. Margaret Read (Infinity Pharmaceuticals, USA) presented sobering data from a phase II trial in pancreatic cancer, in which Hh has been implicated in characteristic stromal hyperplasia. This trial was stopped prematurely due to inferior outcomes in patients receiving Gemcitabine in combination with a Smo inhibitor (Saridegib). A trial of Vismodegib in colon cancer was also reported as negative last year. These studies highlight how much we still have to learn. Despite the progress made in understanding the molecular mechanism and biological function of this fascinating pathway, there are still questions to answer, and it is anyone’s guess as to what twists and turns the story will take before the next hedgehog meeting.

ACKNOWLEDGEMENTS
We thank the speakers who agreed to have their work cited here and we apologize to those we were unable to discuss because of space limitations.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

REFERENCES

Rajat Rohatgi is at the Department of Medicine & Oncology, Stanford University School of Medicine, USA. E-mail: rrohatgi@stanford.edu James Briscoe is at the MRC National Institute for Medical Research, London, UK. E-mail: jbrisco@nimr.mrc.ac.uk

EMBO reports (2012) 13, 580–583; published online 12 June 2012; doi:10.1038/embr.2012.79