

to improve its production and secretion.

DUALITY OF INTEREST

None declared.

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- 1 Maeda K *et al.* *Biochem Biophys Res Commun* 1996; **221**: 286–289.
- 2 Scherer PE *et al.* *J Biol Chem* 1995; **270**: 26746–26749.
- 3 Hu E *et al.* *J Biol Chem* 1996; **271**: 10697–10703.
- 4 Nakano Y *et al.* *J Biochem (Tokyo)* 1996; **120**: 803–812.
- 5 Ouchi N *et al.* *Circulation* 2000; **102**: 1296–1301.
- 6 Ouchi N *et al.* *Circulation* 1999; **100**: 2473–2476.
- 7 Fruebis J *et al.* *Proc Natl Acad Sci USA* 2001; **98**: 2005–2010.
- 8 Yamauchi T *et al.* *Nat Med* 2001; **7**: 941–946.
- 9 Berg AH *et al.* *Nat Med* 2001; **7**: 947–953.
- 10 Danforth E Jr. *Nat Genet* 2000; **26**: 13.
- 11 Shulman GI. Cellular mechanisms of insulin resistance. *J Clin Invest* 2000; **106**: 171–176.
- 12 Weyer C *et al.* *Diabetologica* 2000; **43**: 1498–1506.
- 13 Hotta K *et al.* *Arterioscler Thromb Vasc Biol* 2000; **20**: 1595–1599.
- 14 Shapiro L, Scherer PE. *Curr Biol* 1998; **8**: 335–338.
- 15 Kissebah AH. *Proc Natl Acad Sci USA* 2000; **97**: 14478–14483.
- 16 Spiegelman BM, Flier JS. *Cell* 2001; **104**: 531–543.

Metastatic medulloblastoma— therapeutic success through molecular target identification?

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Metastatic medulloblastoma is a malignant tumor of the developing CNS with little hope for a permanent cure. A report by McDonald *et al* offers alternatives by identifying potential therapeutic targets using expression array analysis.^{1,2}

The last three decades have seen impressive advances in the successful treatment of many common childhood malignancies such as acute lymphoblastic leukemia. This success has unfortunately not been paralleled for malignant brain tumors.³ The most common of these, the cerebellar medulloblastoma, exhibits a tremendous metastatic potential: up to 50% of patients present with metastatic disease at the time of diagnosis (see Figure 1).⁴ While the spread of malignant brain tumors is usually limited to the CNS, medulloblastomas can aggressively metastasize into extraneural tissues.⁵ Due to its prognostic rel-

evance, the metastatic status is used for stratification of patients into risk groups.³

The treatment of patients with standard risk tumors, ie those without metastases has been rather successful with survival rates of up to 78%.^{4,6} In contrast, the cure of metastatic disease has until recently been limited to single cases.

In metastatic medulloblastoma standard chemotherapy regimens combined with neuraxis radiotherapy have shown only temporary response. In younger children radiotherapy is associated with unacceptable neurotoxicity and attempts at delaying the time to radiotherapy are often futile. Survival rates for this group of patients have been close to zero and any relapse has been associated with an inevitably fatal outcome.⁴

This grim scenario has to some extent changed with the introduction of high dose chemotherapy protocols supported by autologous stem cell rescue.⁷

Even though promising, all of the currently successful treatment options for high-risk medulloblastoma are associated with neural and neuroendocrine side effects. Many follow-up studies demonstrate a tremendous decline in quality of life among survivors of medulloblastoma.⁸

To avoid toxicity to the developing brain, therapeutic avenues targeting the underlying molecular lesions have to be explored. The search for the underlying genetic lesions in medulloblastoma has been long, however, knowledge has come only slowly.² Genomic changes, such as the formation of an isochromosome 17q and a loss of heterozygosity distal to 17p13.1 (distal to the *TP53* locus) have been known for over 10 years,⁹ however a proposed tumor suppressor gene in the region has not yet been identified. Table 1 recognizes some of the genes that have been identified in recent years as showing genetic differences between normal (cerebellar) and medulloblastoma tissue.^{2,9}

Recently medulloblastomas have been extensively analyzed by novel genome-wide screening methodologies ranging from comparative genome hybridization over methylation scans to expression profiling.^{1,2,9,10} Expression profiling has stirred high hopes among clinicians and scientists alike as it can rapidly identify expression differences in multiple genes, which then may serve as potential therapeutic targets.

MacDonald *et al* compared

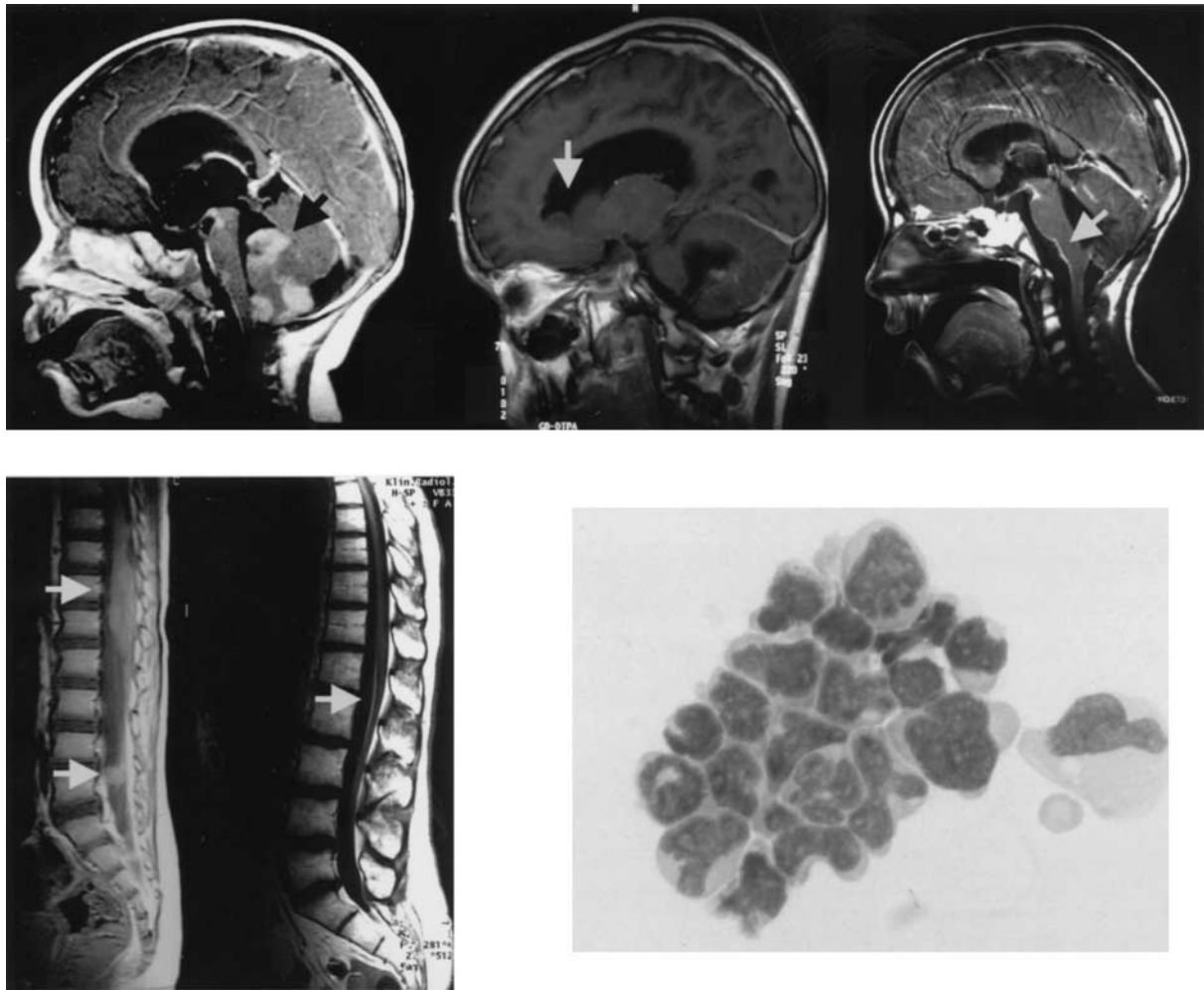


Figure 1 The many faces of metastatic medulloblastoma. The top panel shows sagittal T1-weighted contrast enhanced MRIs of three children with primary metastatic medulloblastoma. Metastases had been demonstrable at diagnosis in all shown cases. From left: nodular tumor in the cerebellum of a 4-year-old; nodular supratentorial metastases on the floor of the ventricles of an 8-year-old; 'sugar-coat' leptomeningeal spread of medulloblastoma in a 10-year-old. Note that in the last two cases the MRIs were taken after the primary tumor had been resected from the cerebellum. Bottom panel from the left: Sagittal contrast T1-weighted MRI in a 5-year-old shows nodular metastases of the spinal cord; 'sugar-coat' leptomeningeal disease in the spinal cord of a 20-year-old; cytopsin preparation of medulloblastoma cells in the CSF of a 1-year-old with disseminated medulloblastoma at diagnosis. (MRIs courtesy of Professor Heindel, Department of Clinical Radiology, University Hospital of Muenster, Germany).

expression arrays of 10 metastatic medulloblastomas with 13 non-metastatic tumors.¹ A total of 85/1992 transcripts were differentially expressed in metastatic tumors, 59 of these up- and 26 downregulated. Most of the upregulated genes play fundamental roles in mechanisms important to metastasis, including angiogenesis, growth factor signaling, cell-adhesion and invasion. Two of the upregulated genes, platelet derived growth factor alpha (*PDGFRA*) and secreted protein acidic and rich in cysteine (*SPARC*) were further analyzed, since they implicated a novel

pathway in metastasis of medulloblastomas. Surprisingly, differential protein expression in metastatic vs non-metastatic tumors could be established using immunohistochemistry and the authors found that blocking of the PDGFA signal or its downstream effector pathways *in vitro* inhibited cell adhesion and migration, two key components of metastatic spread.

This study is a milestone in medulloblastoma research in several ways. In contrast to previous approaches using unselected tumors comparing them to 'normal' tissue, MacDonald *et al* used

specifically metastatic tumors and compared them to non-metastatic ones. Medulloblastoma is a rare tumor affecting up to 6/million children in the US each year. Thus as most studies in the field, this one also suffers from the rather low sample numbers. Furthermore these data represent a thus far unique resource for future pharmacogenetic research in medulloblastomas with many surprises still to be uncovered.

Intriguingly none of the previously described 'prognostic' genes *MYC*, *TRKC* and *ERBB* show any upregul-

Table 1 Genes that are mutated or show expression differences between medulloblastoma and normal cerebellum

Gene	Locus	Comment
<i>PTCH</i>	9q22.1–q31	Homologue of the <i>drosophila</i> segment polarity gene <i>patched</i> . Mutations are found in the nevoid basal cell carcinoma syndrome (NBCCS); most mutations were detected in the desmoplastic subtype of medulloblastoma.
<i>TRKC</i>	15q25	<i>Tyrosine kinase receptor C</i> . Receptor for neurotrophin 3, a member of the nerve growth factor family. High mRNA expression correlates in all studies with a favourable prognosis.
<i>APC</i>	5q23	<i>Adenomatous polyposis coli</i> gene. Mutations are found in the BTPS (brain tumor polyposis syndrome, Turcot's syndrome), which is associated with an increased frequency of medulloblastoma. Mutations in sporadic medulloblastomas are rare.
<i>DMBT1</i>	10q23	<i>Deleted in malignant brain tumors 1</i> . Homozygous deletions in several cases of medulloblastoma found; no studies on single bp-mutations published to date.
<i>NEUROD1,2,3</i>	2q32, 17q12, 5q23–q31	Transcription factors of the bHLH-family with a role in neuronal differentiation. Expression restricted to medulloblastomas. <i>NEUROD3</i> <i>NEUROG</i> expressed primarily in metastatic tumors.
<i>PAX5</i>	9p13	<i>Paired box gene 5</i> . Upregulation in medulloblastomas; no expression in developing cerebellum.
<i>SSTR2</i>	17q24	<i>Somatostatin receptor 2</i> . Upregulated in medulloblastomas as compared to normal cerebellum, where the receptor is mainly expressed in the EGL.
<i>VIPR1/2</i>	3p22, 7q36.3	<i>Vasoactive intestinal peptide receptors 1 and 2</i> . High expression in medulloblastomas. Treatment of cell lines expressing the receptors results in growth inhibition.
<i>CTNNB1</i>	3p22–p21.3	<i>β-Catenin</i> . Activating mutations of this protooncogene have been reported at low frequency in medulloblastomas.
<i>MYC</i>	8q24	Gene amplification and mRNA overexpression are in some studies associated with an unfavourable outcome.
<i>ERBB2</i>	17q21.1	Amplification and overexpression of this oncogene are in some studies associated with an unfavourable prognosis.

ation.^{2,3} This is in clear contrast to published data and deserves further validation in larger sample sets.

In the future it will be highly desirable to combine molecular analysis modalities. For instance several of the identified overexpressed genes are located on chromosome 7 (eg *HOX1*, *RPA* . . .), which is often present in three copies in medulloblastomas. A combination approach may help to determine a rank order of target genes, by separating primary from secondary lesions.

The finding of overexpression of *PDGFRA* and other downstream effectors of the *RAS* protooncogene pathway is a most remarkable point. *PDGFRA*, a receptor tyrosine kinase, has previously been implied in the adult counterpart of medulloblastomas, glioblastomas, and indeed oral treatment of mice with implanted glioblastoma cells using a tyrosine kinase inhibitor (STI 571) inhibited growth.¹¹ Molecular therapeutics, like STI 571 or the *ras*-pathway inhibitors, have become fashionable due to their limited toxicity and a potentially high specificity.¹² The expression of *PDGFRA* had previously been examined in medulloblastomas with no

clear finding. Additionally overexpression does not reflect the functionality of the receptor and may not suffice as an indicator for *in vivo* treatment responses.¹³ Inhibition of the downstream pathway of *PDGFRA* (the MAPK) pathway might also be an oversimplistic approach, as an abundance of stimuli other than *PDGFRA* transmit their signals through this pathway.¹⁴

Nevertheless this study demonstrates that we are not far from individualized treatment protocols. Soon molecular lesion profiles will be established for individual tumors and clinicians may then adjust their therapeutics according to the profile. Unfortunately, metastatic medulloblastomas may take a little longer. For one, most of the molecular lesions against which the currently available drugs are targeted were elucidated 10–20 years ago, when virtually nothing was known on the molecular pathology of medulloblastoma. Secondly, it is rather likely that some of the key features of a medulloblastoma molecular lesion profile are undiscovered and will differ from adult tumors.

Despite the increasing research activities on metastatic medulloblastoma it will take years until solid mol-

ecular profiles will be available. Until then we welcome every potentially efficient therapeutic as an adjunct to current regimens.

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- MacDonald TJ *et al.* *Nat Genet* 2001; **29**: 143–152.
- Frühwald MC. In: Schwab M (ed). *Encyclopedic Reference of Cancer*. Springer: Berlin, 2001.
- Packer RJ *et al.* *Neuro-Oncol* 1999; **1**: 232–250.
- Zeltzer PM *et al.* *J Clin Oncol* 1999; **17**: 832–845.
- Campbell AN *et al.* *Cancer* 1984; **53**: 974–981.
- Kortmann RD *et al.* *Int J Radiat Oncol Biol Phys* 2000; **46**: 269–279.
- Strother D *et al.* *J Clin Oncol* 2001; **19**: 2696–2704.
- Palmer SL *et al.* *J Clin Oncol* 2001; **19**: 2302–2308.
- Biegel JA. *Neurooncology* 1999; **1**: 139–151.

- 10 Frühwald MC *et al.* *Oncogene* 2001; **20**: 5033–5042.
- 11 Kilic T *et al.* *Cancer Res* 2000; **60**: 5143–5150.
- 12 Adjei AA. *J Natl Cancer Inst* 2001; **93**: 1062–1074.
- 13 Black P *et al.* *Pediatr Neurosurg* 1996; **24**: 74–78.
- 14 Schlessinger J. *Cell* 2000; **103**: 211–225.

Feeling below PAR: proteinase-activated receptors and the perception of neuroinflammatory pain

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Interplay between the nervous and immune systems has been the subject of research for several decades. One important aspect of this work is neuronal signaling in inflammatory states, particularly the direct responsiveness of neurons to immune mediators. Multiple techniques have been utilized to understand the increasingly complex physiology and functional role(s) of the plethora of proteins expressed at sites of inflammation, in sensory ganglia and central sites of sensory afferent termination. Consequent to the recent explosion in pharmacogenomics, the number of proteins with as yet undefined function has exponentially increased. This has created the 'luxury' of an over-abundance of potential drug targets, necessitating rational and focussed validation studies.¹ From an analgesic drug discovery perspective, the identification of receptors or channels expressed exclusively by sensory nociceptive neurons makes these attractive targets; hypothetically, a drug working through such a receptor or channel population would block the transmission of nociceptive signals in a manner unlikely to be blighted with centrally-mediated side effects. Currently available analgesics typically act non-selectively at ion channels (for

example, sodium channel blockers) or at neurotransmitter receptors, typically of the G-protein-coupled receptor (GPCR) superfamily (for example, opioids). Though efficacious, they have considerable adverse effect liability. A highly restricted distribution to sensory neurons has not yet been observed for many ion channels or GPCRs, although the capsaicin receptor, VR1, a ligand-gated cation channel, and SNS/PN3, a voltage-gated Na channel are notably localized to sensory ganglion neurons.^{2,3} The recent discovery of the involvement of the GPCR, proteinase-activated receptor-2 (PAR2) in the generation of hyperalgesia, and the observation that its mechanism of action is through sensory neuropeptide regulation is therefore interesting and potentially important.⁴

The PAR family has four members, which are self-activated by innate tethered ligands following proteinase-mediated cleavage of the extracellular amino terminal domain of the receptor.⁵ The proteinases involved in this activation are more commonly associated with protein degradation and include thrombin (cleaves PAR1, PAR3 and PAR4), trypsin (PAR2 and PAR4) and tryptase (PAR2). The evidence for a role of PARs, and particularly PAR2, in inflammation is convincing. For example, activation of PAR2 leads to

smooth muscle relaxation, leukocyte marginalization and infiltration, increased vascular permeability, systemic hypotension and bronchoconstriction (for a review, see Vergnolle *et al*⁵). There is also good reason to suggest an emerging function for PAR2 in neurogenic inflammation. For example, PAR2-immunoreactivity has been demonstrated on enteric neuronal, endothelial and epithelial cells and PAR2 and PAR1 are expressed on primary afferent neurons.⁵ Sixty percent of dorsal root ganglia (DRG) neurons express PAR2-immunoreactivity, significant percentages of which also express calcitonin gene-related peptide (CGRP) and substance P (SP), the two major neuropeptides contained in nociceptive C-fibers innervating superficial laminae of the spinal cord. Activation of these PARs causes rapid intracellular neuronal Ca²⁺ mobilization.⁶ Trypsin, tryptase and PAR2-selective agonists, corresponding to cognate tethered ligand sequences, cause the release of CGRP and SP from C-fibers in peripheral tissues and in spinal cord.⁶ Finally, CGRP₁ and NK₁ receptor antagonists inhibit PAR2 agonist-induced edema. In conclusion, PAR2 agonists mediate neurogenic inflammation via CGRP and SP release and local release of proteinases (eg mast cell tryptase) may result in activation of neuronal PAR2, thereby exacerbating extravasation and edema.

In their most recent publication, Vergnolle *et al*⁴ have demonstrated, using several approaches, the functional complexity of processes activating sensory afferents during noxious stimulation. PAR2 agonists reduced paw withdrawal latencies in thermal and mechanical hyperalgesia tests by up to 64%, as did intraplantar administration of trypsin and tryptase. Confirmation that these were PAR2-specific phenomena was obtained by the lack of effect on withdrawal latencies of the above pharmacological manipulations in PAR2-deficient mice.