REVIEW

Malignant pheochromocytoma: current status and initiatives for future progress

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Abstract

Pheochromocytomas are rare catecholamine-producing neuroendocrine tumors that are usually benign, but which may also present as or develop into a malignancy. Predicting such behavior is notoriously difficult and there are currently no curative treatments for malignant tumors. This report follows from a workshop at the Banbury Conference Center, Cold Spring Harbor, New York, on the 16th–18th November 2003, held to review the state of science and to facilitate future progress in the diagnosis and treatment of malignant pheochromocytoma. The rarity of the tumor and the resulting fragmented nature of studies, typically involving small numbers of patients, represent limiting factors to the development of effective treatments and diagnostic or prognostic markers for malignant disease. Such development is being facilitated by the availability of new genomics-based tools, but for such approaches to succeed ultimately requires comprehensive clinical studies involving large numbers of patients, stringently collected clinical data and tumor samples, and interdisciplinary collaborations among multiple specialist centers. Nevertheless, the well-characterized hereditary basis and the unique functional nature of these neuroendocrine tumors provide a useful framework that offers advantages for establishing the pathways of tumorigenesis and malignancy. Such findings may have relevance for understanding the basis of other more common malignancies where similar frameworks are not available. As the relevant pathways leading to pheochromocytoma are established it should be possible to take advantage of the new generation of drugs being developed to target specific pathways in other malignancies. Again the success of this will require well-designed and coordinated multi-center studies.

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Introduction

Pheochromocytomas are tumors arising from chromaffin cells, mainly of the adrenal gland, that synthesize, store, metabolize, and usually but not always secrete catecholamines (Manger & Gifford 1996). Most are benign and curable by surgical resection, but some are clinically malignant (Lehnert *et al.* 2004). Although the prevalence of malignancy is commonly cited at about 10%, other estimates suggest rates of between 5–26% depending on how malignancy is defined, with lower or even higher values in certain patient groups depending on the underlying mutation (Goldstein *et al.* 1999, Edstrom Elder *et al.* 2003, Gimenez-Roqueplo *et al.* 2003).

Currently, there is no effective cure for malignant pheochromocytoma. There are also no reliable histopathological methods for distinguishing benign from malignant tumors. Instead, malignancy requires evidence of metastases at non-chromaffin sites distant from that of the primary tumor. Although extensive invasion of adjacent tissues can be considered an indicator of malignant potential, local invasiveness and malignant disease are not necessarily associated. The presence of metastases provides the only currently widely accepted means to define malignant pheochromocytoma.

Because there is no cure for malignant pheochromocytoma, nor reliable prognostic or histopathologic diagnostic markers of malignancy, establishing the pathways of tumorigenesis and malignancy in pheochromocytoma represent important objectives that can take advantage of the well-characterized functional nature and genetic background of these tumors and the wealth of information

available about chromaffin cell biology. Apart from development of useful diagnostic and prognostic markers and effective therapies, the findings so obtained might also have broader implications for other malignancies. However, due to the rarity of the tumor, clinical studies about pheochromocytoma suffer from a fragmented nature and usually involve too small a number of cases to reach conclusive results. This undoubtedly contributes to the relatively poor state of funding for clinical research about pheochromocytoma (Table 1) and consequently also limits progress for new diagnostic or prognostic markers and treatments for malignant disease.

In July 2003, Drs James Watson and William Manger met at Cold Spring Harbor Laboratory to discuss the need to differentiate malignant from benign pheochromocytomas on a molecular basis. The workshop held between the 16th and 18th November 2003 at the Banbury Conference Center, Cold Spring Harbor brought together a select group of experts in the field to present data and discuss the current state of knowledge and research about malignant pheochromocytoma. The workshop focused on applications of new genomics-based tools for establishing the pathways involved in the development of pheochromocytoma and for identifying molecular targets for diagnosis, prognosis, and treatment of malignancy in patients with these neuroendocrine tumors. An important objective of the workshop was to establish a consortium of investigators for a coordinated and concerted evidence-based approach to future studies of pheochromocytoma.

Such a consortium approach is already underway for studies of neuroblastoma, which although closely related to pheochromocytoma and overall almost as rare, is one of

Table 1 Pheochromocytoma and neuroblastoma statistics.

	Neuroblastoma	Pheochromocytoma
Annual incidence (per million)*	10.4	3–8
Hereditary contribution (%)	1	> 20
Average age at diagnosis (year)	1.4	42
Mortality due to malignancy (%)†	35	15–26
US extramural federally funded projects§		
2003 Total grants	34	4
2003 New grants	11	0
2004 Total grants	28	2
2004 New grants	6	0

*The annual incidence of pheochromocytoma is not precisely known, but the high prevalence (0.05%) of pheochromocytomas found in autopsy series (McNeil *et al.* 2000) indicates that the tumor is under-diagnosed and that the annual incidence is likely to be higher than indicated.

†Malignancy in pheochromocytoma is almost always fatal so the statistics for this tumor represent the prevalence of malignancy in pheochromocytoma, which is not precisely known. In neuroblastoma, survival varies depending on whether tumors are low risk (98% survival in 35% of tumors), intermediate risk (90–95% survival in 15% of tumors), or high risk (30–40% survival in 50% of tumors).

§Numbers of US federally funded projects involving research on pheochromocytoma and neuroblastoma were determined from a search of the CRISP database (http://crisp.cit.nih.gov/) using the search terms 'pheochromocytoma' or 'neuroblastoma' in both the project title and the abstract. NIH intramural projects are excluded.

the most common and often devastating solid tumors of childhood and which, in contrast to pheochromocytoma, receives some funding (Table 1). In neuroblastoma, as in pheochromocytoma, there are aggressive and relatively benign forms of the tumor. As in pheochromocytoma, these different forms of neuroblastoma are not easily diagnosed or distinguished at an early stage when medical intervention would be most beneficial. Investigators working on neuroblastoma and pheochromocytoma were therefore brought together at the Banbury workshop. Apart from serving as a model for future concerted studies about pheochromocytoma, interactions between investigators in the two fields might yield insights into possible common treatments and pathways responsible for differences in aggressive behavior of both types of tumors. This report provides an update on the current status of research on malignant pheochromocytoma as presented by various participants at the meeting. Also outlined are some considerations, directions, and goals for future studies of benign and malignant pheochromocytoma.

Current therapy

Metastatic disease in pheochromocytoma may be present at the time of initial diagnosis or may only become evident after surgical removal of the primary tumor, usually within 5 years, but sometimes 16 or more years later (Baba *et al.* 1985, Tanaka *et al.* 1993, Lenders *et al.* 2002). Because there is currently no effective cure for malignant pheochromocytoma, most treatments are palliative, but in some cases may reduce tumor burden and prolong survival. Without treatment the 5-year survival is generally less than 50% (John *et al.* 1999). The course, however, can be highly variable with occasional patients living more than 20 years after diagnosis (van den Broek & de Graeff 1978, Yoshida *et al.* 2001).

As reviewed at the Banbury workshop by William Young, once malignancy is diagnosed, therapy is generally directed at controlling blood pressure, but may also include tumor debulking. Hypertension and catecholamine-dependent symptoms can be controlled with α-adrenergic receptor blockade followed by β-adrenergic receptor blockade. Levels of circulating norepinephrine in patients with extensive disease can be extraordinarily high. In such patients consideration should be given to the potentially cytotoxic effects of catecholamines on the myocardium. Inhibition of catecholamine synthesis with α-methyl-paratyrosine (Demser) in exceptional circumstances may be useful in patients with high circulating levels of catecholamines (Decoulx *et al.* 1987, Lehnert *et al.* 2004). The significant side-effect profile of α-methyl-paratyrosine, however, limits the dosage and duration of therapy.

Surgery for malignant pheochromocytoma is rarely curative, but resection of a primary mass or metastases can reduce exposure of the cardiovascular system and organs to toxic levels of circulating catecholamines (Mishra *et al.* 2000). Surgery may also be appropriate for lesions present in life-threatening or debilitating anatomical locations (Nonaka *et al.* 2000). Surgical debulking may also be used before radio- or chemotherapy, but whether this offers any true benefits has not been assessed by any randomized prospective trial. Alternatives to surgical resection include external beam radiation, cryoablation, radiofrequency ablation, transcatheter arterial embolization, chemotherapy, and radiopharmaceutical therapy (Takahashi *et al.* 1999, Pacak *et al.* 2001). Chemotherapy with a combination of cyclophosphamide (Cytoxan), vincristine (Oncovin), and dacarbazine (DTIC-Dome) provides partial remission and improvement of symptoms in up to 50% of patients with malignant pheochromocytoma (Averbuch *et al.* 1988). Usually, however, improvement only lasts for 1 to 2 years. Radiopharmaceutical therapy, using high doses of ^{131}I-meta-iodobenzylguanidine (^{131}I-MIBG), which is transported into the cell via the cell membrane norepinephrine transporter present on most neoplastic chromaffin cells, provides an alternative palliative therapy that can also be effective in temporarily reducing tumor burden and symptoms.

As discussed at the Banbury workshop by Barry Shulkin, therapeutically intended doses of ^{131}I-MIBG have some efficacy in treating malignant pheochromocytoma. Small numbers of patients have been treated using widely varying protocols (Krempf *et al.* 1991, Shapiro *et al.* 1991, Troncone *et al.* 1991, Loh *et al.* 1997, Rose *et al.* 2003, Safford *et al.* 2003). Overall, about 75% of patients treated with ^{131}I-MIBG show improvement in symptoms, 50% have reductions in hormonal activity, and 22% show objective tumor responses. Complete remissions are rare, and progressive disease following ^{131}I-MIBG treatment is common (Schlumberger *et al.* 1992). As a single agent, ^{131}I-MIBG has limited efficacy in treating malignant pheochromocytoma. Its use in combination with other cytotoxic agents, as is currently being studied in patients with neuroblastoma, may result in additional benefit (Sisson *et al.* 1999).

Molecular genetics

Advances in molecular genetics continue to underscore the importance of hereditary factors in the development of pheochromocytoma and propensity to malignancy. Although most cases of pheochromocytoma are sporadic, a significant proportion occur secondary to several hereditary syndromes (Table 2): von Hippel-Lindau (VHL) disease due to mutations of the *VHL* gene,

Table 2 Hereditary pheochromocytoma: facts and figures.

	Gene				
	VHL	RET	NF1	SDHD	SDHB
Frequency in 'sporadic' tumors (%)*	6–10	1–5	unknown	2–8	4–9
Predisposition to malignancy (%)	3	<3	11	<2	66–83
Tumor catecholamine phenotype[†]	NE	E	E	unknown	unknown
Adrenal disease	++	++	++	+	+
Extra-adrenal disease	+	−	+	++	++

*Frequencies of germ-line mutations in apparently sporadic pheochromocytoma and predispositions to malignancy are derived from several sources of data (Walther *et al.* 1999; Aguiar *et al.* 2001; Neumann *et al.* 2002; Bryant *et al.* 2003; Bauters *et al.* 2003; Gimenez-Roqueplo *et al.* 2003).

[†]Tumor catecholamine phenotypes are designated as either epinephrine-producing (E) or predominantly norepinephrine-producing (NE).

++; +; −, Relative likelihoods of adrenal or extra-adrenal disease from high to low.

multiple endocrine neoplasia type 2A and 2B (MEN 2) due to germline mutations of the *RET* gene, neurofibromatosis type 1 (NF1) due to mutations of the *NF* gene, and familial paraganglioma and/or pheochromocytoma syndromes caused by mutations of genes for members of the succinate dehydrogenase family (*SDHB*, *SDHC*, and *SDHD*) (Bryant *et al.* 2003). As genetic screening has become more widely employed, the proportion of cases of pheochromocytoma due to the above mutations has increased above the commonly reported 10% value. Recently, Neumann *et al.* (2002) reported in a population-based study that 24% of apparently sporadic pheochromocytoma patients have such germline mutations, most frequently in *VHL* (10%) and nearly equally frequently (each 5%) in *RET*, *SDHB* and *SDHD* genes. Similarly, from a review of hospital-based studies, Bryant *et al.* (2003) reported that more than 20% of cases of apparently sporadic pheochromocytoma are associated with one of the above four mutations.

As discussed at the workshop by Patricia Dahia, there remain some familial pheochromocytoma syndromes for which the primary genetic defect is still unknown. Also, the genetic basis of the majority of sporadic pheochromocytomas remains largely uncharacterized. Somatic mutations in the genes involved in hereditary pheochromocytoma occur only infrequently in sporadic tumors (Hofstra *et al.* 1996, Brauch *et al.* 1997). By genome-wide scan analysis it now appears that a novel locus in chromosome 2 might account for some remaining cases of familial pheochromocytoma. From a preliminary pilot series of tumors, somatic mutations at this same locus may also be responsible for a significant number of cases of sporadic pheochromocytoma.

Propensity for malignancy in hereditary pheochromocytoma syndromes is highly variable (Table 2). In multiple endocrine neoplasia type 2A, where pheochromocytomas almost always have an adrenal location,

progression to malignancy is rare (Chevinsky *et al.* 1990). In contrast, in familial paraganglioma/pheochromocytoma syndromes due to mutations of the *SDHB* gene, there appears to be a greater risk of extra-adrenal tumors and malignancy (Gimenez-Roqueplo *et al.* 2003). Extra-adrenal tumors also occur in carriers of *VHL* gene mutations and are frequent in those with SDHD mutations. However, in these groups the risk of malignancy is low. Comparisons of the molecular pathways activated in these and other hereditary pheochromocytoma syndromes, which involve differences in malignant potential, should therefore prove useful in understanding the crucial pathways leading to malignancy.

As presented by Graeme Eisenhofer, comparisons of gene expression profiles in MEN 2A and VHL-associated pheochromocytomas revealed activation of hypoxia-driven angiogenic pathways in VHL tumors. This included increased expression of many genes that have been shown in other studies to be over-expressed in malignant compared with benign pheochromocytomas (Salmenkivi *et al.* 2001*a,b*, Favier *et al.* 2002, Khorram-Manesh *et al.* 2002, Salmenkivi *et al.* 2003). Despite this, pheochromocytomas in VHL syndrome have a low rate (~3%) of malignancy. The commonalities of gene expression in malignant and VHL-associated pheochromocytomas appear instead to reflect the biochemical phenotype common to these tumors. Malignant and VHL-associated pheochromocytomas produce predominantly norepinephrine and usually have an exclusively noradrenergic biochemical phenotype, whereas MEN 2 tumors produce both epinephrine and norepinephrine (Rao *et al.* 2000, Eisenhofer *et al.* 2001, van der Harst *et al.* 2002). Sporadic, VHL, and malignant pheochromocytomas with a noradrenergic phenotype all show increased expression of the gene for endothelial Per-Arylhydrocarbon receptor nuclear translocator-Sim (PAS) domain protein 1 (Hif-2α) compared with benign hereditary and sporadic tumors that

produce epinephrine (G Eisenhofer *et al.* 2004). Extra-adrenal pheochromocytomas (paragangliomas) also usually produce exclusively norepinephrine and tend to be more aggressive and likely to metastasize than tumors arising in the adrenal glands which more often produce epinephrine (Brown *et al.* 1972, Kimura *et al.* 1984, John *et al.* 1999, van der Harst *et al.* 2002, Edstrom Elder *et al.* 2003).

As presented by Ronald de Krijger, differences in genetic profile between benign and malignant pheochromocytomas can be observed by comparative genomic hybridization (Dannenberg *et al.* 2000). This genome-wide analysis technique compares normal and tumor DNA by hybridization of differentially labeled DNA to normal human chromosomes and computer-aided analysis of over- or under-representation of either fluorochrome. In this way, it was shown that several regions of loss (8p, 18p) and gain (5p, 7p, 12q) occurred significantly more often in malignant than in benign pheochromocytomas. The main drawback of comparative genomic hybridization, the relatively low resolution, will be overcome by the advent of genome-wide and chromosome arm-specific DNA microarrays, which offer much higher resolution and can pinpoint chromosomal areas of interest for further detailed studies.

The aforementioned differences in tumor genotype and phenotype in benign and malignant pheochromocytomas indicate the importance of considering tumor location, genetics, and biochemical characteristics in proteomics or DNA microarray studies of molecular pathways responsible for malignancy. Comparisons of malignant versus benign pheochromocytomas should therefore include well-defined subgroups of hereditary and sporadic norepinephrine- and epinephrine-producing tumors, and adrenal and extra-adrenal paragangliomas. In studies that involve extra-adrenal paragangliomas, appropriate reporting of anatomic location and other factors, as outlined elsewhere, is essential (Lack *et al.* 2003). Attempts to distinguish paragangliomas associated with sympatho-chromaffin tissue, that produce catecholamines, from those associated with parasympathetic tissue, that do not produce significant amounts of catecholamines, would also be useful.

As outlined at the workshop by David Smith, studies employing proteomics or DNA microarray technology require large numbers of well-characterized tumor samples and access to all relevant clinical data so that appropriate groupings and comparisons can be made. Associated additional technologies such as comparative genomic hybridization can be useful for tracking revealed pathways to candidate genes responsible for tumorigenesis and malignancy. For a rare disease such as pheochromocytoma, such studies are probably best undertaken on a multicenter basis with stringent guidelines for consistent and comprehensive clinical data collection, tissue procurement, tumor banking, and analysis and follow-up of data and patients.

Diagnostic and prognostic markers

As emphasized throughout the workshop, the pathologic distinction of benign and malignant pheochromocytomas and extra-adrenal paragangliomas remains a diagnostic challenge. Nevertheless, as reviewed by Arthur Tischler, several studies have attempted to distinguish benign from malignant pheochromocytoma using histopathological classifications that incorporate retrospective assessment of growth characteristics, cellularity, vascular invasion, capsular invasion, mitotic figures, necrosis and other variables (Kimura & Sasano 1990, Linnoila *et al.* 1990, van der Harst *et al.* 2000, Thompson 2002). Such grading systems require assessment of many variables and as yet have not been applied prospectively for diagnostic or prognostic purposes.

Hendrik Lehnert presented evidence that molecular markers, such as expression of human telomerase reverse transcriptase and heat shock protein 90 (HSP90) (Boltze *et al.* 2003, Elder *et al.* 2003), might provide alternative methods for distinguishing malignant from benign pheochromocytoma (Table 3). Other possible molecular markers include secretogranin II-derived peptide (Yon *et al.* 2003) and numerous factors associated with angiogenesis (Salmenkivi *et al.* 2001a,b, Favier *et al.* 2002, Khorram-Manesh *et al.* 2002, Zielke *et al.* 2002, Salmenkivi *et al.* 2003). Thomas Giordano indicated that a truly reliable predictor of malignant behavior would likely only be achieved through use of a combination of molecular markers. The availability of a transcriptional signature for malignant pheochromocytoma and paraganglioma derived from gene expression profiling studies might permit development of diagnostic tests for this purpose (Giordano 2003). The consensus of the meeting participants was that transcriptional profiling, at least in the short-term, should be compared with and probably integrated with histopathological criteria proposed in the earlier studies. Carefully designed prospective studies will be required to provide convincing documentation of the reliability of these tests. Assessment of the prognostic value of such tests requires careful patient follow-up over many years.

Apart from assessment of tumor tissue samples, there also exists the possibility to establish malignancy or potential for malignancy from markers in biological fluids. As reviewed by David Goldstein, high plasma and urinary levels of dihydroxyphenylalanine and dopamine, the immediate precursors of norepinephrine, are biochemical

Table 3 Molecular markers of malignant pheochromocytoma.

	Reference
Heat shock protein 90	Boltze *et al.* (2003)
Human telomerase reverse transcriptase	Boltze *et al.* (2003), Elder *et al.* (2003)
Vascular endothelial growth factor	Favier *et al.* (2002), Zielke *et al.* (2002), Salmenkivi *et al.* (2003)
Vascular endothelial growth factor receptor 2	Favier *et al.* (2002)
Hypoxia inducible factor 2-alpha	Favier *et al.* (2002)
Cyclooxygenase-2	Salmenkivi *et al.* (2001*b*)
Tenascin C	Salmenkivi *et al.* (2001*a*)
N-cadherin	Khorram-Manesh *et al.* (2002)
Secretogranin II-derived peptide EM66	Yon *et al.* (2003)

hallmarks that may characterize malignant pheochromocytoma (Anton *et al.* 1967, Goldstein *et al.* 1986, John *et al.* 1999, Januszewicz *et al.* 2001, van der Harst *et al.* 2002). While suggestive of a dedifferentiated state that might be associated with malignancy, these markers do not, however, accurately discriminate benign from malignant pheochromocytomas. Karel Pacak presented data, based on a proteomics study, indicating that the patterns of expression of peptides and proteins in serum could distinguish patients with solitary apparently benign pheochromocytomas from those with metastases. An accurate diagnostic test based on such patterns would represent a major advance in guiding patient management.

Currently, identification of metastases requires imaging studies, such as whole body computed tomography (CT) or magnetic resonance imaging (MRI) scans, ^{131}I-metaiodobenzylguanidine (^{131}I-MIBG) scintigraphy, ^{131}I-MIBG single photon emission computed tomography (SPECT), and bone scans (Shapiro *et al.* 2001). Other nuclear imaging technologies such as ^{18}F-fluorodeoxyglucose positron emission tomography (PET) (Neumann *et al.* 1996) and ^{111}In-octreotide SPECT (Tenenbaum *et al.* 1995, van der Harst *et al.* 2001) are also available, but do not have the same level of sensitivity and specificity as imaging agents such as MIBG that target the cell membrane norepinephrine transporter present on most pheochromocytomas. MIBG imaging, however, only detects 85–90% of pheochromocytomas and sensitivity may be lower for malignant pheochromocytoma. Recently Ilias *et al.* (2003) reported that 7 out of 16 patients with malignant pheochromocytoma had negative ^{131}I-MIBG scans indicating a sensitivity of 56%. Use of PET scanning with the imaging agent, ^{18}F-6-fluorodopamine, which also targets the norepinephrine transporter, provided a more sensitive method for identifying metastatic pheochromocytoma. Presently, however, the technology has limited availability and, as in MIBG imaging, still fails to detect occasional dedifferentiated tumors that lack expression or express low levels of the norepinephrine transporter. In such patients ^{18}F-fluorodeoxyglucose or ^{111}In-octreotide can be useful (Tenenbaum *et al.* 1995).

PET scanning with ^{18}F-fluorodopa provides another alternative that in preliminary studies has yielded encouraging results (Hoegerle *et al.* 2002).

Another problem associated with present day diagnosis and treatment of malignant pheochromocytoma is a lack of consensus about when imaging studies should be carried out to detect or exclude metastatic disease. Although by no means unanimous, the view supported by most of the participants at the workshop was that malignant disease should be considered at the time of initial diagnosis and before surgical resection. This contrasts with a recent report suggesting that the routine use of MIBG scintigraphy in the uncomplicated patient before operation may be unnecessary (Miskulin *et al.* 2003). When there is increased risk for malignant disease (e.g. paraganglioma or > 6 cm in diameter adrenal pheochromocytoma) or increased risk for more than one catecholamine-secreting tumor (e.g. paraganglioma patients), MIBG imaging should always be considered preoperatively. Whether or not MIBG imaging is used routinely, it must also be considered that the modality has imperfect sensitivity for excluding all cases of malignancy. Thus, biochemical testing should always be repeated after recovery from surgical resection of a primary mass to exclude any remaining disease or metastases.

Because metastases may be microscopic at the time of initial surgery, and therefore may not present as malignant disease until many years later, biochemical screening should continue at yearly intervals (Baba *et al.* 1985, Tanaka *et al.* 1993, Lenders *et al.* 2002). Again, however, there is a lack of consensus regarding the duration of follow-up and in what form follow-up screening should take. Some have indicated that biochemical testing alone is insufficient and that follow-up examinations should include imaging studies (Morikawa *et al.* 2001). Although it is generally agreed that follow-up should be long-term, it has recently been suggested that follow-up may not be necessary for all patients with a resected solitary tumor (Edstrom Elder *et al.* 2003). Certainly the accumulating evidence indicates that continued screening may be most important in patients with

paragangliomas or tumors judged by existing histopathological criteria to be at risk for malignancy.

The above issues for patient management will depend on further advances in diagnosis and treatment. With improved diagnostic markers it should become possible to assess more effectively the presence of malignancy. With improved prognostic markers it should also become possible to ascertain whether a tumor is truly pathologically benign and does not require follow-up, or possesses malignant potential and requires regular patient monitoring. With this and further developments in treatment it may even be possible to target patients at risk for malignancy with prophylactic therapies. Certainly, as more effective treatments are developed it should become possible to establish a consensus about the importance of an earlier diagnosis of malignant disease, when therapies might be more likely to be curative.

Model systems

As discussed at the Banbury workshop by Arthur Tischler, model systems are essential for the determination of mechanisms and pathways responsible for tumorigenesis and malignancy in pheochromocytoma and for the development and testing of new treatments. A problem here is lack of an established human pheochromocytoma cell line. Currently, the only relevant cell lines are those from pheochromocytomas in rodents. These lines have been difficult to establish because pheochromocytoma cells usually cease proliferating in cell culture. Current models include the rat PC12 line, established in 1976 (Greene & Tischler 1976), and several more recently developed mouse pheochromocytoma ('MPC') lines from neurofibromatosis knockout mice (Powers *et al.* 2000). Advantages of the PC12 line include an enormous amount of accrued data and a remarkably stable phenotype for 28 years. A disadvantage is that the PC12 line is representative of only a single cell from one tumor. In contrast, multiple MPC lines with somewhat differing characteristics have been derived from separate tumors. These lines all over-express non-mutated *ret*, similar to many human pheochromocytomas, making them highly relevant for studies of ret signaling (Powers *et al.* 2002).

There also exist several animal models of pheochromocytoma. In rats, numerous pharmacologically diverse and usually non-mutagenic substances readily induce pheochromocytomas. Some agents that cause rat pheochromocytomas in long-term toxicity studies (e.g. the anti-hypertensive drug, reserpine) can be shown to increase chromaffin cell proliferation in short-term experiments. Tischler and colleagues hypothesize that chromaffin cell mitogenesis may set the stage for genetic damage by endogenous mutagens produced during catecholamine metabolism. Potential mutagens include quinones, semiquinones, reactive oxygen species, and short-lived aldehyde intermediates produced by oxidative deamination of catecholamines. Some disadvantages of the rat model are that the genetic basis of pheochromocytomas in that species and relevance to human disease are unknown.

In contrast to rats, pheochromocytomas in mice arise with increased frequency in several transgenic or knockout models involving genes associated with human pheochromocytomas. These include the neurofibromatosis knockouts described above and MEN 2B transgenic models (Smith-Hicks *et al.* 2000). In addition to the germline abnormalities, common denominators between human and mouse pheochromocytomas include similar profiles of secondary genetic changes detected by comparative genomic hybridization. These include deletions of mouse chromosome 4 that are homologous to the common human 1p deletion (Benn *et al.* 2000, You *et al.* 2002), and deletions of mouse chromosomes 4 and 9 that are homologous to human 3p and 3q deletions (J F Powers, A S Tischler, M Mohammed & R Naeem, unpublished observations). Disruptions of the gene for phosphatase and tension homologue deleted on chromosome 10 (PTEN), a tumor suppressor located on mouse chromosome 4, appear in particular to predispose to spontaneous development of pheochromocytomas and may represent a candidate for human disease (Podsypanina *et al.* 2001, You *et al.* 2002).

The use of nude mice to host pheochromocytoma cells from human tumors and animal cell lines, provides another model that may be particularly useful in assessing the efficacy of new treatments (Zielke *et al.* 1998). Using this model, antibodies to vascular endothelial growth factor have been shown to inhibit angiogenesis in PC12 xenografts (Zielke *et al.* 2002). In another study, halofuginone, an inhibitor of collagen synthesis and extracellular matrix deposition, was found to markedly reduce tumor size in xenografts of human VHL pheochromocytomas (Gross *et al.* 2003). Since PC12 and MPC cell lines were developed using xenografts, this system may also be the most promising for development of much-needed human pheochromocytoma cell lines.

Future therapies and new initiatives

Reports of occasional patients who show apparent complete remission of malignant disease after ^{131}I-MIBG therapy (Rose *et al.* 2003) provide an incentive for continued studies that seek to improve targeting of this radiopharmaceutical to pheochromocytoma cells (Fig. 1). Similarly, objective response rates to
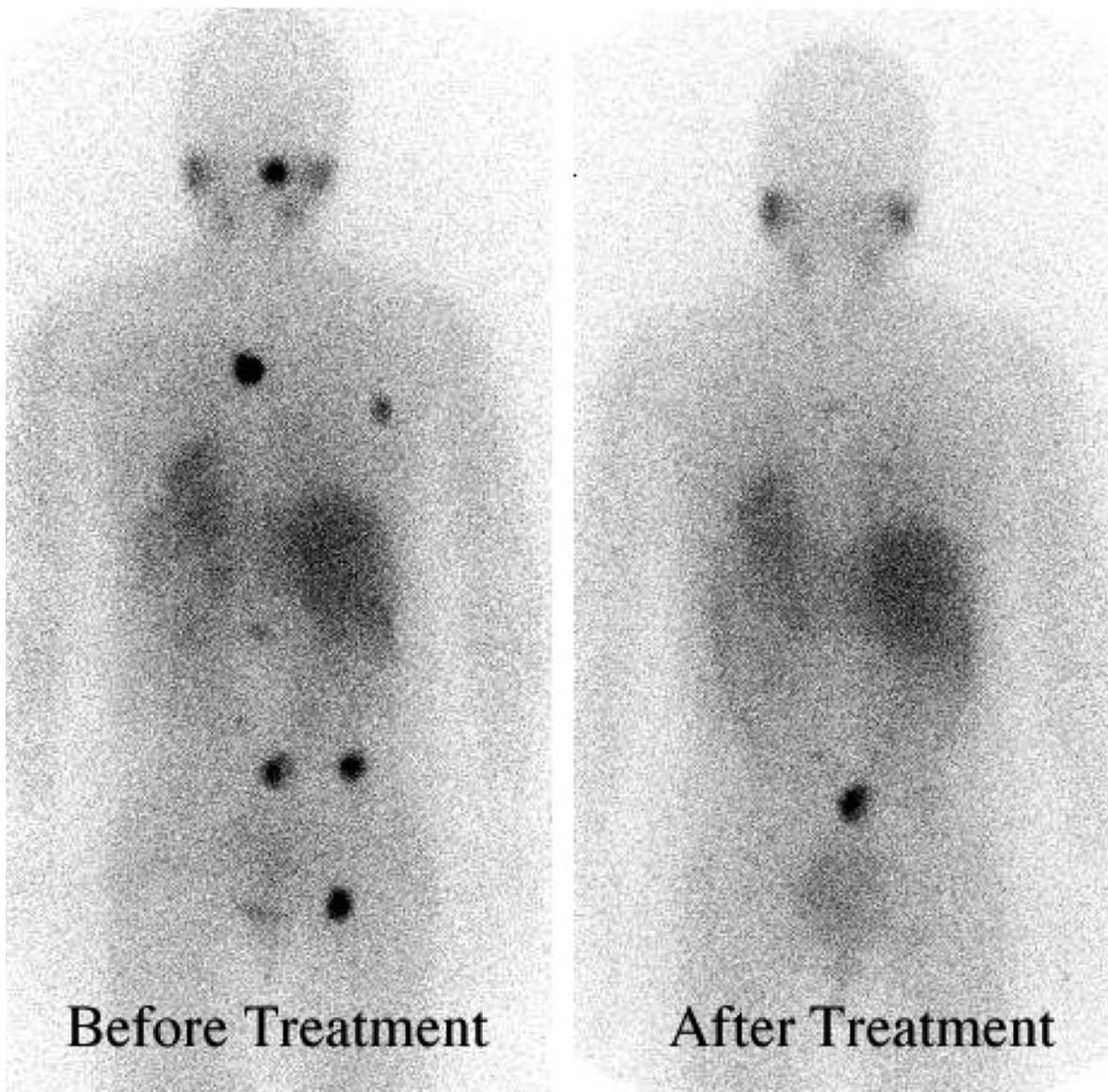
Figure 1 ^{131}I-MIBG scintigraphs of a patient with malignant pheochromocytoma before and after high-dose MIBG treatment based on the protocol of Rose *et al.* (2003). Images kindly provided by the Department of Nuclear Medicine, University of Düsseldorf.

^{131}I-MIBG therapy in heavily pretreated neuroblastoma patients may be as high as 30–50% (Matthay *et al.* 1998, Kang *et al.* 2003), and this is superior to essentially every other novel agent studied in the setting of a relapsed high-risk tumor. Ongoing investigations have been designed to combine ^{131}I-MIBG with conventional chemotherapy, and/or to increase the dose intensity of ^{131}I-MIBG by providing for multiple infusions. This has been an extremely well-tolerated therapy with hematopoietic toxicity being dose limiting, but abrogated with peripheral blood stem cell support (Matthay *et al.* 1998, Kang *et al.* 2003).

Accumulation and retention of ^{131}I-MIBG in pheochromocytoma tumor cells depends on expression of catecholamine transporters on the cell surface and in chromaffin granules within which catecholamines are stored (Jaques *et al.* 1987, Kolby *et al.* 2003). Thus, better and more toxic substrates for these transporters or strategies designed to increase the expression of transporter systems on tumor cells before ^{131}I-MIBG therapy offer approaches to improve therapeutic targeting. Such approaches, which to date have been mainly limited to experimental model systems, include norepinephrine transporter gene transfer (Cunningham *et al.* 2000,

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Boyd *et al.* 2001) and increasing expression of the norepinephrine transporter using cisplatin (Armour *et al.* 1997) and combinations of interferon-gamma, tumor necrosis factor-alpha, and retinoic acid (Montaldo *et al.* 1996). A clinical treatment protocol, based on such studies, has been approved at the NIH, Bethesda, MD, USA, but has been on hold due to institutional funding and staffing limitations. At the Children's Hospital of Philadelphia, John Maris reports similar problems for their clinical trials to treat neuroblastoma, which together with high costs and lack of appropriate commercial sources of ^{131}I-MIBG constrains availability of the therapy to only a few of many eligible pediatric patients. Future progress in these and other clinical therapeutics trials requires encouragement of a multidisciplinary team approach at the institutional level with an adequate level of support to ensure that all eligible patients have access to new experimental treatments as these become available. Identification of a suitable vendor at the US national level would be useful for trials involving ^{131}I-MIBG.

Because neuroendocrine tumors, including pheochromocytomas, express a number of subtypes of somatostatin receptors, administration of analogs of somatostatin have been proposed as another treatment for malignant pheochromocytoma (Wiseman & Kvols 1995). Although some initial small scale studies have indicated reduction in tumor catecholamine production (Invitti *et al.* 1993) or symptomatic improvement (Kopf *et al.* 1997), this has not been confirmed by other groups (Lamarre-Cliche *et al.* 2002) and may only be useful in subgroups of tumors with membrane-associated somatostatin type 3 receptors (Mundschenk *et al.* 2003). Nevertheless, as discussed at the workshop by Hendrik Lehnert, combined treatment with novel somatostatin analogs and ^{131}I-MIBG may confer additional benefit over ^{131}I-MIBG treatment alone.

Development of other more effective targeted therapies for malignant pheochromocytoma can be expected to take advantage of the wealth of new drugs being developed in response to advances in the elucidation of pathways responsible for other cancers. Use of DNA microarray and proteomics technologies for understanding the pathways contributing to benign and malignant pheochromocytoma should be useful for guiding the choice of the most appropriate of these agents for future therapeutic trials.

Already there are suggestions of possible targets in malignant pheochromocytoma for new classes of anti-cancer drugs being developed in response to improved understanding of pathways involved in other malignancies (Table 3). Over-expression of HSP90 in malignant pheochromocytomas (Boltze *et al.* 2003), in particular, indicates one promising therapeutic target for a new class of anticancer drugs being developed to inhibit this protein

(Maloney & Workman 2002). HSP90 is now understood to function as a molecular chaperone that maintains the folding and conformation of proteins crucial in regulating the balance between degradation and synthesis of cell signaling proteins, including many involved in multiple oncogenic pathways. Such proteins include the human telomerase reverse transcriptase, which also shows increased expression in malignant pheochromocytoma (Boltze *et al.* 2003, Elder *et al.* 2003). Should HSP90 be proven to be involved in the transition from a benign to malignant pheochromocytoma phenotype, then new inhibitors of the protein, such as 17-allylamino, 17-demethoxy-geldamycin (Sausville *et al.* 2003), may be of value as treatments for the malignancy. Findings from several groups that malignant pheochromocytomas are characterized by increased expression of factors associated with angiogenesis (Table 3) suggest other pathways that may respond to new anti-angiogenic drugs currently in development or approved by the Food and Drug Administration for specific types of cancer.

Even with the advent of new therapeutic targets and drugs it remains likely that a combination of therapies will be required for effective treatment. Given the heterogenous nature of pheochromocytomas, it is also likely that for best therapeutic results, treatments may have to be tailored according to differences in underlying pathways. Developing such approaches will benefit from the opportunities available from the new genomic and proteomic methodologies and other developments associated with new drug discovery technologies, such as high throughput screening of drug candidates.

The current unfolding of the NIH roadmap (Zerhouni 2003) makes this a time of flux in research priorities and a movement towards future advances in medical research that will take advantage of the tremendous information available in the post-genome era. This is also a pivotal time-point to move forward from past and present methods and procedures for diagnosis, localization, and treatment of pheochromocytoma to research into novel methodologies based on the new pathways to discovery (Table 4). As outlined in the new roadmap, taking advantage of the new technologies available in the post-genome era is best undertaken by multidisciplinary research teams involving centers and individuals with the necessary expertise. Importantly, research about pheochromocytoma already includes such a platform, involving interactions among clinicians and investigators in diverse fields, including endocrinology, human genetics, clinical chemistry, radiology, nuclear medicine, surgery, pathology, and oncology. This already existing multidisciplinary platform can be broadened to further take advantage of new technologies and, due to the rare nature of the tumor, should also better bring together various

Table 4 Malignant pheochromocytoma — past, present, and future

	Past	Present	Future
Biochemical diagnosis	Urinary VMA, catecholamines, and total metanephrines. Plasma catecholamines	Plasma and urinary fractionated metanephrines and catecholamines	Plasma markers derived from proteomics and RNA technology
Tumor localization	X-ray	CT, MRI, MIBG, bone scans	Functional and molecular imaging with PET
Pathological diagnosis	Chromaffin reaction	Histopathological markers, presence of metastases	DNA microarrays and molecular markers
Genetic predisposition	Family history	Mutation analysis	High-throughput sequencing, microarray-based genotyping
Post-surgical testing and management	Biochemical testing 2–6 weeks post-surgery	Biochemical testing 2–6 weeks post-surgery. Annual screening of all patients	Biochemical testing 2–6 weeks post-surgery. Annual screening of selected patients based on prognostic molecular markers, prophylactic therapies in select patients
Therapy	Adrenergic blockers, α-methyl-paratyrosine and use of other drugs for symptomatic relief	Surgical debulking, chemotherapy, ^{131}I-MIBG radiotherapy, radiofrequency ablation, cryoablation, chemoembolization	Tumor sensitization to ^{131}I-MIBG radiotherapy, pathway-specific molecular targeting, cancer vaccines, gene therapy

VMA, vanillylmandelic acid.

groups of investigators involved in pheochromocytoma research. This will be particularly important as new treatments and drugs become available.

Rather than the fragmented approach typical of previous trials, such as those that have assessed the therapeutic efficacy of ^{131}I-MIBG, it is desirable for future trials of novel targeted therapies for malignant pheochromocytoma to be carried out in a more concerted and coordinated manner. The support and establishment through the NIH roadmap of new centers for bioinformatics, computational biology, database libraries and other tools provides added incentive for investigators working on pheochromocytoma to pull together for a more focused and concerted approach to find solutions to the outstanding problems associated with this tumor.

The Pheochromocytoma RESearch Support ORganization (PRESSOR) was established as an outcome of the November 2003 workshop at Cold Spring Harbor to facilitate improved evidence-based multidisciplinary approaches for research on pheochromocytoma. The consortium (http://www.pressor.org) has over 100 members worldwide, including many leaders in established fields as well as in new or emerging fields of research. One of the goals of the consortium is to develop and implement effective treatments for malignant pheochromocytoma. Another goal is to establish new diagnostic and prognostic tests for discriminating benign from malignant and other forms of pheochromocytoma. The consortium also seeks to create platforms for the exchange of information on pheochromocytoma research among investigators, physicians, and patients. It will also support, through evidence-based studies, the establishment of consensus-derived guidelines for effective biochemical diagnosis, localization, management, and treatment of benign, malignant, and other forms of pheochromocytoma. Through a focused and coordinated approach, we envisage that members of the consortium will be able to satisfy these goals more rapidly than would otherwise be possible.

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