Title
Immortalized neurons derived from tumors in transgenic mice

Permalink
https://escholarship.org/uc/item/45g9r0jk

Journal
Trends in Neurosciences, 14(1)

ISSN
0166-2236

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Publication Date
1991

DOI
10.1016/0166-2236(91)90170-Y

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Peer reviewed
Tissue-specific enhancer elements have been mapped for many genes, but remain largely a mystery for genes that are expressed specifically in neurons. Why should this be the case? There is a practical reason: for cells other than neurons, tissue-specific gene expression has been generally studied in transient expression assays, which depend on the transfection of DNA into large numbers of recipient cells (usually established cell lines). These assays quantify the activity of a reporter gene, often encoding an enzyme such as bacterial chloramphenicol acetyltransferase (CAT), whose expression is directed by flanking DNA sequences of the gene being studied. In the past, neurons have been virtually impossible to study in this way: it is difficult to obtain pure populations of neurons in primary culture, especially in sufficient quantity to make transient expression assays worth performing, and neurons are also relatively intractable to DNA transfection. As there are almost no cell lines that represent specific types of neurons, in vitro studies of gene expression that is specific to the nervous system have been limited to genes that are expressed in tumors such as gliomas, neuroblastomas and pheochromocytomas, which occur with reasonable frequency and from which cell lines can be established (for example, see Refs 2, 3).

The alternative to using cell lines is to study tissue-specific gene expression in transgenic mice. This approach has advantages and disadvantages: the organism itself does all the necessary tissue controls, but the approach is costly and, more importantly, it is slow. The time from initial egg transfer to tail blot is at least six weeks (this can be reduced to three weeks if polymerase chain reaction techniques are used to detect the presence of the transgene), and it then takes another seven to eight weeks before the next generation can be tested to determine if germ-line transformation has been achieved. In contrast, transfection assays of transient gene expression take only a few days.

Despite the fact that generating a transgenic mouse is a slow process, it might nonetheless offer the most direct route from the identification of a gene that is expressed specifically in a particular type of neuron, to in vitro studies of gene regulation in that cell type. This is because transgenic mice can now be used to generate cell lines from particular types of neurons. In two recent studies, different groups have used relatively long stretches (approximately 2 kilobases) of 5' flanking DNA sequences from two genes expressed in the nervous system to direct oncoprotein expression in transgenic mice. In both cases, transgenic mice developed tumors in the appropriate regions of the nervous system and in both cases the investigators have been successful in establishing cultured cell lines with the properties of neurons from these tumors (Fig. 1).

The use of transgenic mice to target tumorigenesis to a specific cell type was first demonstrated by Hanahan. Mice carrying a transgene that consisted of the 5' flanking sequences of the rat insulin II gene fused to the SV40 early region encoding large and small tumor antigens (Tag) specifically expressed Tag in the beta cells of the endocrine pancreas. Tumors developed with a high frequency (1–2% of the islets progressed to tumors) and retained at least one important property of the beta cells: they expressed insulin, so much so that transgenic mice bearing tumors died prematurely of severe hypoglycemia. Baetge and colleagues used the same strategy to target catecholaminergic cells of the adrenal gland and retina, using 2 kilobases of the 5' flanking region of the human phenylethanolamine N-methyltransferase (PNMT) gene to direct Tag expression in transgenic mice. Targeted Tag expression led to the formation of adrenal and eye tumors. In a more recent study, they describe the establishment of a neuronal cell line from an eye tumor of one of these transgenic mice. Cultured cells from this tumor (RT-1 cells) have a neuronal morphology (phase-bright cell bodies and extensive processes), and express neuronal proteins such as GAP-43 and all three neurofilament subunits. The cells are also immunoreactive with two monoclonal antibodies that stain amacrine cells of the rat retina, VCl.1 and HPC-1 (Refs 10, 11). However, RT-1 cells do not express the enzymes that are characteristic of catecholaminergic neurons: tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis, and PNMT, the terminal enzyme in this pathway. The failure of RT-1 cells to express TH is perhaps not surprising; there is a population of PNMT-immunoreactive amacrine cells in the retina that lack detectable TH, and these might very well be the cells that were immortalized by the strategy used by Baetge and colleagues.

Lack of PNMT expression is more disturbing, however, since it is presumably the PNMT promoter/enhancer that directs the continuous expression of Tag in these cells. The eye tumors themselves are reported to lack catecholaminergic properties, so the failure of RT-1 cells to express PNMT is probably not a result of culture conditions. One possibility is that the cells immortalized in the eye tumors are not the PNMT-expressing amacrine cells of the retina at all. Ectopic expression of chimeric genes in transgenic mice has been observed, and might be the result of novel expression specificities dictated by the juxtaposition of cis-acting regulatory sequences from unrelated genes (see Ref. 13). Alternatively, as the authors suggest, Tag expression might somehow suppress expression of the endogenous PNMT gene. If this is the case, however, suppression must be specific to retinal neurons, since the authors note that adrenal cell lines derived from tumors in the same
transgenic mice express both PNMT protein and mRNA. Furthermore, it is known that, in vivo, the adrenergic phenotype (and by inference, PNMT expression) is not itself incompatible with cell proliferation since during post-natal development of the adrenal gland in the rat, mitotic figures are observed in adrenaline-storing cells.

It should be noted, however, that expression of catecholamine biosynthetic enzymes may be regulated differently in the central and peripheral nervous systems. For example, several studies of TH expression during development have shown that TH is expressed in CNS neurons only after their final mitosis, whereas, in the PNS, dividing cells can express TH. The question of whether or not the retinal tumors in the hPNMT-Tag transgenic mice result from ectopic Tag expression might be resolved when the authors examine the expression of PNMT in Tag-positive cells during the early stages of transgene expression. If PNMT is expressed in Tag-positive cells, then this result will be consistent with the suppression hypothesis, and will make the ectopic expression hypothesis less likely. In any case, the fact that RT-1 cells have at least some of the properties of differentiated amacrine cells holds promise for future studies of amacrine cell-specific gene expression. Mellon and colleagues have immortalized neurons that secrete gonadotropin-releasing hormone (GnRH) by targeting expression of Tag with 2.3 kilobases of the rat GnRH gene 5′ flanking region. Two out of nine transgenic mice that were produced in this way developed tumors of the anterior hypothalamus and one of these tumors was the source of an established cell line. Unlike the case where the PNMT promoter/enhancer was used to direct Tag expression, both the hypothalamic tumor and the cell line (GT) derived from it express GnRH mRNA and protein. In addition, depolarization can regulate hormone release in these cells: veratridine, which opens Na+ channels, stimulates release of GnRH from GT cells and this release can be blocked by pre-treatment of the cells with tetrodotoxin.

Like RT-1 cells, GT cells have a neuronal morphology and express general markers of neuronal differentiation such as neuron-specific enolase and the 68 kDa neurofilament protein. GT cells also express the mRNAs of two synaptic proteins, VAMP-2, a vesicle-associated protein expressed in the hypothalamus, and SNAP-25, a protein associated with the synaptic membrane, as well as chromogranin B, a neuroendocrine secretory vesicle protein. Interestingly, while the authors report that the neuronal morphology of these cells is more pronounced after serum deprivation, which also inhibits cell division, the expression of neural markers is unaffected by this manipulation. Apparently, proliferation and expression of the differentiated neuronal phenotype are not incompatible, at least for this class of neuron.

What lies ahead? Certainly, both the RT-1 and GT cell lines should provide defined systems by which investigators can learn more about the molecular and cellular biology of hypothalamic and retinal neurons. For two specific classes of neurons these cell lines now allow experiments using the sorts of techniques that require biochemical quantitation of pure populations of cells — subcellular fractionation, protein and nucleic acid purification, and isolation of molecules involved in signal transduction cascades, to name but a few. Will it be possible to immortalize any type of neuron for which an appropriate targeting regulatory sequence can be identified? There might be certain restrictions on this technique: for both PNMT and GnRH, it appears that expression can occur in cells before they withdraw from the cell cycle. It may be that oncogene expression must be targeted to specific populations of neuronal precursors in order to obtain tumor formation. A useful by-product of future attempts to target tumorigenesis to other types of neurons should thus be an increase in our understanding of the developmental regulation of neuron-specific gene expression, since positive results should only be obtained in cases where conditions allowing transgene expression exist.

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Migraine: a research field matured for the basic neurosciences

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Progress in migraine research has been rapid in recent years, from both the basic science and the clinical perspectives. A new internationally accepted headache classification with operational diagnostic criteria was published in 1988, eliminating much diagnostic uncertainty. More than a decade of study of regional cerebral blood flow (rCBF) has gradually shown a pathognomonic pattern of abnormalities, probably reflecting spreading cortical depression. Recently it has been shown that pain probably arises from excitation of perivascular pial arterial nociceptors. The innervation and receptor mechanisms of pial and extracranial arteries have been worked out in detail both in animal and human. Involvement of calcitonin gene-related peptide (CGRP) and 5-hydroxytryptamine (5-HT) during migraine attacks has been demonstrated. A new and specific 5-HT1D receptor agonist has proved to be highly effective in treating migraine attacks; the release of perivascular peptides during migraine attacks; and the association of 5-hydroxytryptamine (5-HT) and its receptors with migraine pathogenesis.

Improved classification and diagnostic criteria for headache disorders

The first attempt to classify and define headache disorders was made in 1963 by an ad hoc committee of the National Institutes of Health (USA). The definition of migraine read as follows: ‘attacks of headache, widely varying in intensity, frequency and duration etc.’. Such criteria cannot be used consistently, because they do not specify whether one or more of these clinical characteristics should be present, or how often they should be present. Furthermore, diagnostic terms were ambiguous at that time, and were used differently in different countries. If a disease is poorly defined, it is also difficult to study its mechanisms, since not all of the patients under investigation will actually be suffering from the disorder. However, a committee of the International Headache Society has now completed a new headache classification containing operational diagnostic criteria. The headache classification, originally published in English, has also been published in French, German, Italian, Slovenian and Turkish. Translations into Spanish, Portuguese and Japanese are in progress. The classification has been sponsored by the World Federation of Neurology, the Research Group on Migraine and Other Headaches and has, in simplified form, been used by the World Health Organization (WHO) in the most recent International Classification of Diseases (ICD-10). Epidemiological and genetic studies of headache will benefit immensely from the new classification, which will also facilitate pathophysiological and therapeutic studies.

Studies of regional cerebral blood flow (rCBF) during migraine attacks

For more than a century, scientists have debated the importance of vascular and neurogenic (CNS) components in the pathophysiology of migraine. Despite the lack of suitable methods for studying arterial pulsations, arterial diameter or blood flow, Wolff gathered evidence in favour of the vascular hypothesis during the 1940s and 1950s. It was not until the advent of the intra-arterial xenon method that quantitative studies of rCBF could be done during migraine attacks, and it was only with the development of computerized multi-detector systems that measurements with sufficient spatial resolution were obtained. This development has been reviewed previously, and therefore the rest of this article focuses on more recent observations. Early on in an attack of migraine with aura, rCBF is reduced at the posterior pole of the brain; this reduced flow gradually involves larger areas of one