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Minireview

Lessons from polyoma middle T antigen on signaling and transformation: A DNA tumor virus contribution to the war on cancer

Brian S. Schaffhausen [a], Thomas M. Roberts [b,c,*]

[a] Department of Biochemistry, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111, USA
[b] Department of Cancer Biology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115, USA
[c] Department of Pathology, Harvard Medical School, Boston, MA 02115, USA

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ABSTRACT

Middle T antigen (MT) is the principal oncogene of murine polyomavirus. Its study has led to the discovery of the roles of tyrosine kinase and phosphoinositide 3-kinase (PI3K) signaling in mammalian growth control and transformation. MT is necessary for viral transformation in tissue culture cells and tumorigenesis in animals. When expressed alone as a transgene, MT causes tumors in a wide variety of tissues. It has no known catalytic activity, but rather acts by assembling cellular signal transduction molecules. Protein phosphatase 2A, protein tyrosine kinases of the src family, PI3K, phospholipase Cγ1 as well as the Shc/Grb2 adaptors are all assembled on MT. Their activation sets off a series of signaling cascades. Analyses of virus mutants as well as transgenic animals have demonstrated that the effects of a given signal depend not only tissue type, but on the genetic background of the host animal. There remain many opportunities as we seek a full molecular understanding of MT and apply some of its lessons to human cancer.

An introduction to MT and this review

Since its discovery in 1953, polyomavirus has served as a model system for the study of transformation mechanisms and cellular signaling. Murine polyoma's role in signaling research arises from a quirk of evolution that created an extra reading frame in the early region of the virus. The resulting third early protein was designated middle T antigen (MT) on the basis of its apparent molecular weight. Unlike small T (ST) and large T (LT) antigens, which work in transformation primarily by acting on host tumor suppressor proteins such as p53, pRb and protein phosphatase 2A (PP2A), MT acts by activating key proto-oncogenes involved in cellular signaling. In this way MT effectively mimics a constitutively activated receptor tyrosine kinase (RTK). Its primary structure is diagrammed below in Fig. 1. Mechanistically, MT, like an RTK, first binds and activates a tyrosine kinase. In the case of the RTK, a ligand is used to bind a second RTK, thus activating both members of the ligand induced dimer. MT binds and activates a member of the src family of non receptor tyrosine kinases using both a motif on MT and a portion of the PP2A phosphatase as a scaffold. The activated src family kinase in turn phosphorylates MT on key tyrosines generating docking sites for cellular signaling molecules—a process which is conceptually identical to the signaling mechanism of a ligand activated RTK. Notably studies of MT have significantly contributed to our understanding of cellular signal transduction. Thus, the first tyrosine kinase assays were conducted on MT immunoprecipitates as were the key early experiments on PI3 kinase (for another review see Dilworth, 2002). With inhibitors of PI3K now entering the clinic it may soon be possible to credit study of this viral oncogene as contributing to cancer therapy, thus delivering on the early promise of tumor virology to the war on cancer.

In the following review we will examine MT's role in transformation and signaling in more detail. While space does not permit a full historical record of each of these studies, we will attempt to track the history of key parts of the story. After an overview, our narrative will roughly follow the diagram in Fig. 1 from the amino to carboxyl terminus and finish with a short consideration of animal studies.

Overview of MT function and structure

MT is the key to polyomavirus transformation. It is always necessary for virus transformation (Carmichael et al., 1982; Templeton and Eckhart, 1982). MT is in many cases sufficient (Treisman et al., 1981) for transformation of cells in tissue culture. Where it is not sufficient, MT can be complemented by a variety of oncogenes generally thought to act in the nucleus, such as LT (Land, Parada, and Weinberg, 1983), E1A (Ruley, 1983), myc (Land et al., 1983) and p53 (Utermark et al., 2007), as well as ST (Moule et al., 2004; O'Shea and Fried, 2005), which acts on the p53 pathway. The importance of MT in polyomavirus tumor induction is clear (Asselin et al., 1984; Cullere et al., 1998; Freund et al., 1992a, 1992c). When expressed as a transgene, MT causes tumors in a variety of tissues (Aguzzi et al., 1990; Bautch, 1989; Du et al., 2006; Guy et al., 1992; Lewis et al., 2005; Rassoulzadegan et al., 1990; Tehranian et al., 1996). MT has been a

* Corresponding author. Dana-Farber Cancer Institute, Department of Cancer Biology, 44 Binney Street, Boston, MA 02115, USA. Fax: +1 617 632 4770.
E-mail addresses: brian.schaffhausen@tufts.edu (B.S. Schaffhausen), thomas_roberts@dfci.harvard.edu (T.M. Roberts).

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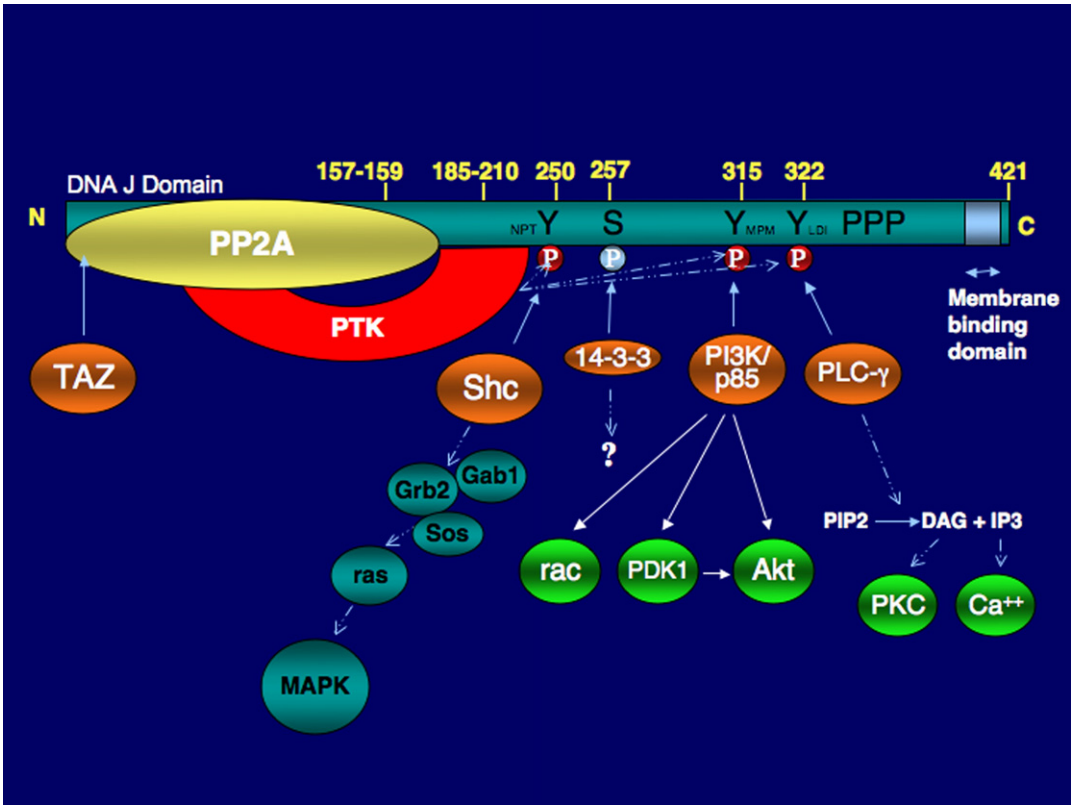

Fig. 1. Landmarks on MT. Sites of interaction of MT with cellular proteins are shown. Each of the signal transducers shown below the middle T sequence is known to be important for transformation. PP2A = protein phosphatase 2A, PTK = src family tyrosine kinase (src, yes, fyn), PI3K = phospholinositide 3-kinase, and PLCγ = phospholipase Cγ1. There can also be interaction between Gab1 and PI3K. PPP represents the proline rich/E349K sequence important for transformation. Simple versions of signaling pathways downstream of Y250, Y315 and Y322 are shown. The light blue area represents the hydrophobic membrane attachment site.

particularly helpful model, because mutations affecting particular associations with host proteins have generally had clear phenotypes in transformation (Cullere et al., 1998; Freund et al., 1992a). By comparison, the effects of mutations of single tyrosine phosphorylation sites on growth factor receptors, such as the PDGFr, have been harder to evaluate.

Besides its role in transformation, MT also plays an important role in polyoma infection (Freund et al., 1992c; Garcea et al., 1989; Templeton et al., 1986). These effects can occur at different levels. A defect in viral assembly associated with altered phosphorylation of VP1, which responds to MT (Li and Garcea, 1994), can be observed in hr-t mutants lacking MT (Garcea and Benjamin, 1983). MT also participates in regulating both viral DNA replication and RNA transcription (Chen and Fluck, 2001; Chen et al., 1995, 2006).

MT has 421 amino acids. Since the T antigens are produced by differential splicing of common early transcripts, MT shares 79 amino acids that represent a DnaJ domain with both LT and ST, as well as an additional 112 amino acids with ST. There is a stretch of 22 hydrophobic residues that represent a membrane anchor sequence at the C-terminus. The ability of MT to transform depends on its association with membranes (Carmichael et al., 1982). MT has no known catalytic activity, but rather functions as a scaffold on which cellular signaling proteins are assembled and activated. It binds the A and C subunits of protein phosphatase 2A (PP2A) (Pallas et al., 1990; Walter et al., 1990). As a result of this association, MT is able to bind protein tyrosine kinases (PTKs) of the Src family (Src, Yes, Fyn) (Cheng et al., 1988a; Courtneidge and Smith, 1983; Horak et al., 1989; Kornbluth et al., 1987). In the PTK complex, MT is phosphorylated on three major tyrosine residues: 315, 322 and 250 (Carmichael et al., 1984; Harvey et al., 1984; Hunter et al., 1984; Schaffhausen and Benjamin, 1981a). Each of these sites represents a connection to a signal generator: 315 to phosphoinositide 3-kinase (PI3K) and one or more additional interacting proteins (Hong et al., 2003; Kaplan et al., 1986; Whitman et al., 1985), 250 to Shc (Campbell et al., 1994; Dilworth et al., 1994) and thence to Grb2 and SOS, and 322 to PLCgamma1 (Su et al., 1995). However, this picture of three tyrosine phosphorylation sites is not complete. Additional minor tyrosine phosphorylation sites may also contribute to MT function (Chen et al., 2006). Finally, serine phosphorylation at 257, which controls association with the 14-3-3 family, affects the ability of MT to cause salivary gland tumors (Cullere et al., 1998). We will discuss each of these interactions and their role in MT signaling and transformation in turn.

MT and PP2A

MT (and ST) binds protein phosphatase 2A (PP2A) (Pallas et al., 1990; Walter et al., 1990). Mutational analysis of MT indicates that sequences near the N-terminus (Glenn and Eckhart, 1995), in the region from 111–120 (Campbell et al., 1995; Glenn and Eckhart, 1993), in the area from 142 through the conserved WFG motif at 157 (Campbell et al., 1995; Martens et al., 1989 and Li and Schaffhausen, unpub.) and through NG59 mutation at 179 (Cheng et al., 1986; Silver et al., 1978) or a deletion of residues 180–190 (Brewster et al., 1997) can all affect PP2A binding. Some of these residues are in cysteine motifs that are expected, based on SV40 ST structures (Chen et al., 2007; Cho et al., 2007) to be involved in zinc binding (Rose and Schaffhausen, 1995). PP2A is one of the major cellular serine/threonine protein phosphatases (Janssens and Goris, 2001; Janssens et al., 2005; Millward et al., 1999; Mumby, 2007). It functions as a trimeric ABC complex, where a scaffold A subunit brings together the catalytic subunit (C) and one of a large family of regulatory B subunits (Fig. 2A). There are two isoforms each of A and C, while there are four B families, each with several members. MT binds to the A and C subunits (Pallas et al., 1990; Walter et al., 1990), displacing or preventing B subunits from binding (Fig. 2B). Since structural analysis shows that SV40 ST

binds to regions of the A subunit involved in B binding (Chen et al., 2007; Cho et al., 2007), the apparent absence of B subunits in Py/PP2A complexes is unsurprising. MT binds both A alpha and A beta isoforms of PP2A, while SV40ST in comparison binds only the A alpha form (Zhou et al., 2003). While the functional consequence of this difference is unclear, mutations in PP2A A beta have been associated with lung and colon cancer (Ruediger et al., 2001; Wang et al., 1998). It also appears that A beta is involved in regulating the RalA GTPase (Sablina et al., 2007).

There is an obvious and critical role for PP2A in MT function. Association of MT with PP2A is required for association with the Src family PTKs (Brewster et al., 1997; Campbell et al., 1995; Ogris et al., 1999), so PP2A$^-$ MT is unable to transform (see the section on Src kinases below). Interestingly, PP2A catalytic activity seems not to be required (Ogris et al., 1997) to promote binding of PTKs. This suggests that PP2A is part of a scaffold that allows src to bind to MT. There has also been a suggestion based on a study of a PP2A binding mutant of MT (Brewster et al., 1997) that PP2A binding affects the intracellular location of MT. However, there is a problem in interpreting this kind of experiment. Because abrogation of the ability of MT to bind PP2A allows hsc70 to bind (Campbell et al., 1995), hsc70 binding might well affect intracellular localization. There is also a report that MT uses PP2A to activate c-jun kinase (Mullane et al., 1998).

The obvious role of PP2A in src recruitment has perhaps led investigators away from detailed examination of the catalytic roles of PP2A in MT action. This is probably short-sighted. PP2A is involved in many cellular processes, including transcription, translation and replication. At least 50 protein kinases are regulated by PP2A (Millward et al., 1999), and it functions as a tumor suppressor (Janssens et al., 2005; Westermarck and Hahn, 2008). Decreasing the activity of PP2A towards myc has been associated with head and neck carcinoma and colon cancer (Junttila et al., 2007). This raises the question of how MT changes the activity of PP2A. There are some hints from ST studies. SV40ST inhibits PP2A activity in many situations (Scheidtmann et al., 1991; Yang et al., 1991). In some situations, such as transformation of kidney cells (Chen et al., 2004), knock-down of specific B subunits mimics some of the effect of SV40ST in causing transformation, while overexpression of B subunits can reverse some of the effect of SV40ST. While there is certainly not enough MT to titrate most or all of the cell's PP2A, even reducing the available pool of subunits to create a situation of haploinsufficiency affects the spectrum of PP2A complexes (Chen et al., 2005). However, it is also important to note that perturbation of PP2A function by SV40ST increases the activity of PP2A towards certain substrates, such as histone H1 (Yang et al., 1991) or the androgen receptor (Yang et al., 2005). Such effects are likely to contribute to the transformed phenotype as well. The idea would be that MT could target PP2A towards specific substrates. There is now evidence that lipin, for example, is a substrate dephosphorylated as a result of its interaction with MT (B. Schaffhausen, unpublished).

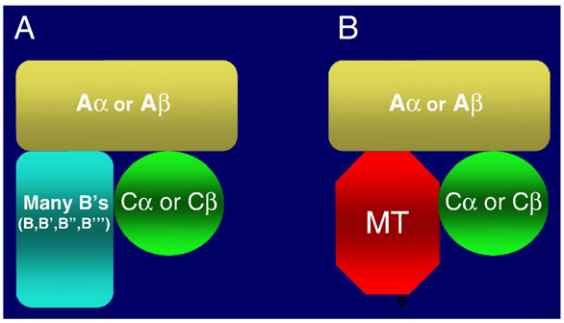

Fig. 2. MT and PP2A:PP2A exists as heterotrimeric ABC complexes. (A) There are two different A subunit scaffolds to which two different catalytic C subunits could bind. There are many different B family regulatory subunits. (B) The binding of PyST (or PyMT) replaces B subunits.

MT and the Src family of tyrosine kinases

As noted in the Introduction, the first tyrosine kinase assays were carried out on MT immunoprecipitates (Eckhart et al., 1979). Only later was it determined that kinase activity associated with MT comes from binding protein tyrosine kinases (PTKs) of the src family (src, yes fyn) (Cheng et al., 1988a; Courtneidge and Smith, 1983; Horak et al., 1989; Kornbluth et al., 1987). However, MT does not associate with all members of the src family. Thus, it fails to bind lck (Louie et al., 1988) or hck (Dunant et al., 1996). The tyrosine kinase activity coming from association with src kinases is critical for transformation (Carmichael et al., 1984; Eckhart et al., 1979; Harvey et al., 1984; Schaffhausen and Benjamin, 1979; Smith et al., 1979). Manipulating the level of tyrosine kinase activity either by regulating MT levels (Raptis et al., 1985) or manipulating src with anti-sense affected the degree of transformation (Amini et al., 1986). It appears that association with particular family members may be differentially important in specific tissues. In Fisher rat cells, it has been estimated that 50–75% of the activity is from src and the remainder from the other kinase complexes (Bolen et al., 1987). In a transgenic MT model of mammary carcinoma, src is the functionally important partner (Guy et al., 1994). For hemangiomas, although single knockouts of src, yes or fyn do not block MT tumor formation, yes knockouts respond much more weakly to MT, with fewer tumors and longer latency (Kiefer et al., 1994; Thomas et al., 1993).

The association with the src family PTKs is unusual in a number of respects. Only small fractions (~10%) of MT and src are in MT/src complexes (Bolen et al., 1987). Furthermore, overexpression of either partner is not effective at driving complex formation (Cook et al., 1990; Piwnica-Worms et al., 1986; Schaffhausen et al., 1987). Finally, the interaction between MT and src is slow, taking hours to complete (Cohen et al., 1990).

Src itself has several domains organized in the following order: an N-terminal myristylation sequence, a unique domain, an SH3 domain, an SH2 domain and a catalytic domain with a C-terminal tail that contains a regulatory phosphorylation site at Y527. Phosphorylation at 527 provides a binding site for the src SH2 that results in restricted activity. MT needs only the catalytic domain and C-terminal sequences for association (Dunant et al., 1996). The C-terminal sequences around the regulatory phosphorylation site are important for binding: a truncation at 516 fails to bind MT (Cartwright et al., 1987), mutations in the C-terminal region abolish binding and v-src that has an altered C-terminus also fails to bind (Cheng et al., 1988b). It appears that additional sequences further N-terminal are also required, since a src–lck fusion, where the C-terminus comes from lck (that itself does not bind MT), nonetheless binds MT (Louie et al., 1988). Dunant et al. have investigated the failure of hck to bind MT (Dunant et al., 1996). Based on their observations on a C-terminal Y501 hck mutant, they concluded that MT was unable to bind to a closed conformation which had a pY/SH2 interaction, but rather would bind to an open configuration. In support of this idea, they presented data arguing that src that had the SH2 domain deleted showed enhanced MT binding.

MT mutations that affect src binding have been found in a number of areas. The primary binding site on MT for binding PTKs has been defined genetically as lying between residues 185–210, with 2 basic motifs followed by an S or T playing important roles (Brewster et al., 1997; Glover et al., 1999). However, other regions also seem to be important to form the MT/PTK complex. Mutations near the N-terminus of MT (Cook and Hassell, 1990; Glenn and Eckhart, 1995; Templeton and Eckhart, 1984b), in the J domain (Whalen et al., 2005), and in the PP2A binding site (Campbell et al., 1995; Schaffhausen and Benjamin, 1979) also disrupt assembly of MT/PTK complexes. In general, these results support the idea that PP2A binding is required

for the recruitment of src, since of the defective mutants only Q37A (Whalen et al., 2005) has been reported to bind PP2A. L5E (Glenn and Eckhart, 1995) is a different kind of exception in that it has been reported to bind a phosphatase, apparently different from PP2A, but still to bind src. Some PP2A inhibition experiments (Glover et al., 1999) and a report suggesting that PP2A might be able to dephosphorylate phosphotyrosine (Cayla et al., 1993) might suggest a catalytic role for PP2A in formation of the MT–PTK complex. However, examination of catalytically dead PP2A C subunit has shown that it can support MT/src complex formation (Ogris et al., 1997).

Src bound to MT is activated (Bolen et al., 1984; Courtneidge, 1985), showing a substantial increase in the V_{max} of kinase activity. Src in the MT complex is also unphosphorylated at the C-terminal 527Y (Cartwright et al., 1986; Courtneidge, 1985). These observations were key to the general understanding of how src kinase activity is negatively regulated (Cartwright et al., 1987; Cooper et al., 1986; Kmiecik and Shalloway, 1987; Piwnica-Worms et al., 1987). At the same time src is phosphorylated on Y416 in MT complexes, a site known to be activating (Cartwright et al., 1986). Interestingly, there also appears to be additional N-terminal sites of src tyrosine phosphorylation in MT complexes (Cartwright et al., 1986; Yonemoto et al., 1985). However, these additional sites (Y138 and Y213) are known to respond to growth factors and may regulate SH3 and SH2 interactions respectively (see Roskoski, 2005 for a review).

In the case of transformation by v-src, there is a dramatic increase in total ptyr in the transformed cells (Hunter and Sefton, 1980). There is not a broad general increase in tyrosine phosphorylation in MT transformed cells (Sefton et al., 1980), although treatment of cells with vanadate, a tyrosine phosphate inhibitor, led to a significant increase over the controls (Yonemoto et al., 1987). Some src targets, such as stat3 (Garcia et al., 1997) seem to be activated by MT, while others, such as p34, do not (Cheng and Chen, 1981). MT itself is perhaps the most important substrate. In the PTK complex middle T is phosphorylated on tyrosine residues, including major sites at 315, 322 and 250 (Harvey et al., 1984; Hunter et al., 1984; Schaffhausen and Benjamin, 1981b). Some phosphorylation is observed at 297 and possibly 258 as well (unpublished). These phosphorylations connect MT to different signaling pathways that will be discussed in turn.

The tyrosine 250 phosphorylation site binds Shc

There is no question that MT is phosphorylated at tyrosine 250 in the MT/PTK complex (Hunter et al., 1984). However, in many respects the role of this residue is enigmatic. In many in vitro assays of transformation, mutation of tyrosine 250 (Markland et al., 1986; Ong et al., 2001; Utermark et al., 2007) as well as mutations in the region amino terminal to 250 (the NPTY motif) (Druker et al., 1990; Druker et al., 1992) have a dramatic effect on transforming ability. As will be discussed below, the 250 site leads to the recruitment of the adaptor Shc (Campbell et al., 1994; Dilworth et al., 1994). In the transgenic mammary tumors induced by MT, mutation of tyrosine 250 also has a profound effect on tumor formation (Webster et al., 1998). Tumor studies in virus-infected animals have also confirmed a role for the 250 site in polyoma tumorigenesis (Bronson et al., 1997; Yi et al., 1997). The frequency of kidney tumors, for example, is dramatically reduced in 250 mutants. On the other hand, there are cells such as human mammary epithelial cells and fibroblasts where mutation of Y250 to F has no effect on transformation (Utermark et al., 2007). Even different clones of F111 rat embryo fibroblasts show different sensitivity to Y250F mutations (Ong et al., 2001). In contrast to the transgenic experiments in the mammary gland, virus experiments in mice with Y250S show little, if any difference, in the frequency of mammary tumors (Bronson et al., 1997; Yi et al., 1997), although there were differences in the morphological types of tumors (Yi et al., 1997). The virus experiments also showed that Y250S mutants could produce larger tumors than wild type, for example in hair follicle tumors that were more than 100× larger in volume than wild type, or produce a greater frequency (2/3 of mice) of tumors such as penile papillomas that are quite rare after infection with wild type virus (Bronson et al., 1997). As in many other situations, such results indicate the importance of context in signaling.

The most immediate and obvious role for the Y250 phosphorylation is to provide a docking site for the ShcA family of adaptors (Campbell et al., 1994; Dilworth et al., 1994). The ShcA family includes three overlapping isoforms of p46, p52 and p66 kDa (Pelicci et al., 1992). p46 and p52 are ubiquitous, while p66 is more restricted and subject to epigenetic regulation (Ventura et al., 2002). All 3 isoforms share a domain structure that has a N-terminal PTB domain and a C-terminal SH2 domain, both of which can potentially bind phosphotyrosine sequences (Schlessinger and Lemmon, 2003), as well as a central proline-rich CH1 region. While the data are clearest for p52, MT appears to bind and affect all 3 family members. The PTB domain is clearly important for binding MT. Shc PTBs bind ΨXNPXpY motifs (van der Geer et al., 1995, 1996a). Mutation of these residues in MT clearly affects its ability to bind and affect Shc phosphorylation as well as transform (Campbell et al., 1994; Druker et al., 1990, 1992). Overexpression of the Shc PTB domain inhibits MT transformation of NIH3T3 cells (Blaikie et al., 1997). However, since MT has also been reported to bind to the Shc SH2 in vitro (Dilworth et al., 1994) and since P248H, but not Y250F, induced a small amount of Shc tyrosine phosphorylation in vivo (Campbell et al., 1994), the possibility of some SH2 interaction cannot be excluded.

Although only a small fraction of p52 is bound to MT in immunoprecipitates, a significant fraction of total p52 is phosphorylated upon MT expression (Campbell et al., 1994). Shc is tyrosine phosphorylated on at least 3 sites, Y317 (Y313 in mouse) (Salcini et al., 1994) and a double phosphorylation site at Y239/Y240 (van der Geer et al., 1996b). Phosphospecific antibodies show that MT affects both of these sites (Jiang, Zhu and Schaffhausen, unpubl.) It has been suggested (e.g. Gotoh et al., 1996) that these two phosphorylation sites have distinct functions, but this issue has never been examined for MT. It is known that expression of Y239F/Y240F Shc, but not Y317F Shc, affects the size of agar colonies of MT transformed cells (Blaikie et al., 1997).

Both the Y317(Y313) site (Salcini et al., 1994) and the Y239/Y240 site (van der Geer et al., 1996b) have been reported to bind Grb2. Each of these sites has been reported to recruit the Grb2 adaptor. It is therefore unsurprising that MT promotes the association of Shc with Grb2 (Campbell et al., 1994; Dilworth et al., 1994) and that Grb2 is found in MT immunoprecipitates (Nicholson et al., 2001). The MT sequence around Y250 has been replaced with Shc sequences (313 or 239/240) known to bind Grb2. This has been done either at the same location or between residues MT 281–284, a region not thought to be important for MT transformation. For transformation of Rat2 cells, insertion of the 239/240 sequence was more potent than the 313 sequence (Nicholson et al., 2001). However, since some substitutions did not fully recapitulate wild type function, even when binding amounts of Grb2 comparable to wild type, the authors concluded that Shc probably does more than interact with Grb2. Replacing the NPTY sequence of MT with a GRB binding sequence (either YVNQ or YYD) stimulated the production of hemangiomas and the introduction of both sequences worked best, with tumor latencies matching that of wild type MT (Ong et al., 2001). In transgenic experiments, overexpressing Grb2 (or Shc) reduced the time needed for tumor onset with MT Y250F from 111 days to 80 days (or 94 days) (Rauh et al., 1999).

The recruitment of Grb2 allows the binding of additional proteins. One of these is the adaptor Gab1 (Nicholson et al., 2001; Ong et al., 2001). Gab1 plays an important role in signaling of a number of growth factors and cytokine receptors (Nishida and Hirano, 2003). Of potential importance, Gab1 is involved in the recruitment of PI3-kinase. This will be discussed further in the next section. Gab1 would also be expected to bind the SHP-2 tyrosine phosphatase and the adaptor Crkl. The binding of Grb-2 leads to the recruitment of SOS

(Nicholson et al., 2001), a Ras exchange factor. This would be expected to lead to Ras activation, which is, in fact observed (Srinivas et al, 1994). Once Ras is activated this should lead to Raf, MEK, ERK activation, and then to p90rsk or transcription factors such as Elk. In fact, little published confirmation of ERK activation by MT is available except in unusual situations such as HL60 cells undergoing differentiation (Yen et al., 1999). There is evidence that Shp2 is also connected to growth factor activation of ERK (Nishida and Hirano, 2003). Whether the Gab1 interaction connects Shp2 regulation of ERK is an open question.

Tyrosine 315 and PI3K

The principle role of tyrosine 315 when phosphorylated is to bind particular isoforms of PI3 Kinase (PI3K). Here we recount in a largely historical fashion how MT allowed identification of PI3 kinases. We also take the liberty of describing the subsequent realization that PI3Ks were a potential drug target in human tumors. The next few years should tell if PI3K inhibition will have therapeutic effects in cancer.

Before the discovery of PI3K, tyrosine 315 was one of the first sites on MT to be examined by site directed mutagenesis with unexpectedly mixed results. Two groups made identical Tyr315 to Phe mutations, and then expressed the mutant proteins in different cell types using different expression systems. When expressed via polyoma virus infection of F111 rat cells, the 315 mutant was almost totally inactive in transformation (Carmichael et al., 1984); when transfected in Rat-1 cells as a plasmid construct expressing only MT, the 315 mutant was almost as effective in transformation as wild type MT (Oostra et al., 1983). Although this difference was never totally resolved, it is likely simply the first case of a common theme in MT transformation and signaling: the importance of cellular context to signaling outcome and requirements. The true significance of Tyr315 could be realized only upon the discovery of its binding partner, PI3K (Whitman et al., 1985).

PI3Ks are a family of lipid kinases distinguished from one another by their substrates, cellular functions and subunit composition. The Class 1A PI3Ks were first studied as a phosphatidylinositol kinase (PIK) activity in partially purified preparations of the oncoprotein $pp60^{v\text{-}src}$ (Sugimoto et al., 1984). A similar activity was found in immunoprecipitates of MT, which by that time was known to function by binding and activating $pp60^{c\text{-}src}$ (Kaplan et al., 1986; Whitman et al., 1985). However, oncogene-associated PIK activity was only a small fraction of the total PIK activity in the cell and, thus, was considered by many to be an artifact of isolation. The importance of the PI kinase was a subject of considerable controversy for some time. Notably, however, the PIK activity was found associated with all transformation competent alleles of MT, but not with a number of non-transforming alleles including deletions or point mutants of Y315 (Kaplan et al., 1986). In time, it was realized that the small fraction of MT-bound PIK activity possessed unique biochemical properties, designated type I PIK, which distinguished it from the bulk of the PIK in the cell (Whitman et al., 1987). The presence of the type I PIK activity in immunoprecipitates of MT was tightly correlated with the presence of an 85 kDa polypeptide (Kaplan et al., 1987). More importantly, both the type 1 PIK and p85 were soon found to be physically associated with a variety of activated receptor tyrosine kinases, and once again genetic experiments, using mutants which failed to bind the PI kinase, suggested that the association was essential for the biological functions of both RTKs and oncoproteins (Escobedo et al., 1991; Kaplan et al., 1987; Varticovski et al., 1989). With the cloning of the gene for p85, it became clear that p85 is an SH2 containing adaptor protein designed to couple the type I PIK to activated RTKs and oncoproteins such as MT via its interactions with specific phosphotyrosine motifs (Escobedo et al., 1991; Hu et al., 1992). In the case of MT this is the sequence phosphoYMPM starting at Tyr315 on MT. Further analysis showed that there were actually 2 genes p85α and p85β, encoding highly similar proteins which both bind MT (Cohen et al., 1990). To date no distinction in the functions of p85α/β have been found for MT, even though knock-out studies have shown the 2 proteins to have distinct roles in development (Fruman et al., 1998). As an aside, we note that the association between MT and the N-terminal SH2 of p85 provided a prototype for establishing specificity of SH2 domains (Songyang et al., 1993) and a structural model that showed how NMR can be used to demonstrate ligand induced conformation intermediates during high affinity binding (Gunther et al., 2002; Mittag et al., 2004). The next flurry of discoveries identified and characterized the p110 catalytic subunits of the type I PIKs. Classical PIKs were known to phosphorylate phosphatidylinositol (PI) on the 4′ position on the inositol ring to generate PI(4)P. However, it was noticed that type 1 PIK associated with MT could use PI(4)P as a substrate (Whitman et al., 1988), suggesting that type1 PIK was not simply phosphorylating the 4′ position on the inositol ring. Instead, the type 1 enzyme was shown to phosphorylate the 3′ position on the inositol ring to generate PI(3,4)P2, thus leading to its renaming as PI3K (Whitman et al., 1988). Purified PI3K associated with RTKs and oncoproteins was found to consist of two subunits: the p85 adaptor plus a catalytic subunit of 110 kDa (Carpenter et al., 1990). With further purification and molecular cloning of PI3Ks, it became clear that there was actually a family of PI3Ks (Hiles et al., 1992; Hu et al., 1993). The family was divided into three groups: Class I PI3Ks, which could use PI, PI(4)P, and PI(4,5)P2 as substrates in vitro; Class II PI3Ks which utilized PI and PI(4)P; and Class III PI3Ks, restricted to PI as a substrate (see reviews by Fruman et al., 1998 and Vanhaesebroeck and Waterfield, 1999). Notably, Class1 enzymes seem to be limited to the use of PI(4,5)P2 as a substrate in vivo. Class I was further divided into three Class IA enzymes, each consisting of the p85 adaptor subunit complexed with one of three p110 catalytic subunits (α, β, and δ) and capable of associating with RTKs and oncoproteins, and Class IB, a single species that features a different subunit composition and is largely confined to leukocytes. The discovery in the late 1990s that firmly established the Class 1A PI3Ks as oncogenes was the finding that p110α had been captured by an avian retrovirus rendering it oncogenic (Chang et al., 1997).

After the discovery of PI3K, it was some time before the downstream signaling targets of PI3K were identified. Over time however it became clear that the role of PIP3 was to lure certain PH (pleckstrin homology) domain containing proteins to the membrane via a direct interaction of the PH domain and PIP3. Among the key targets are the ser/thr kinase PDK1 and its downstream target the ser/thr kinase Akt. Akt in turn phosphorylates a large number of targets regulating cell growth, DNA synthesis, chemotaxis and apoptosis. As might be expected MT can activate Akt (Dahl et al., 1998; Meili et al., 1998; Summers et al., 1998) and Rac (Denis and Schaffhausen, unpublished). Activation of Akt by MT prevents apoptosis (Dahl et al., 1998; Meili et al., 1998). The functional consequences of Akt activation have been demonstrated most elegantly in the MMTV-MT mammary tumor model (see below).

Also activated via a PH domain-containing nucleotide exchange protein is the small GTP binding rac (for a review see Bustelo et al., 2007). Dominant-negative Rac activation blocks transformation of both fibroblasts (Urich et al., 1997) and endothelial cells (Connolly et al., 2000). It appears that Rac activation is necessary for MT to activate c-fos (Chen et al., 1999; Urich et al., 1997).

The discovery that the tumor suppressor PTEN actually works by antagonizing PI3K showed the first direct link between PI3K activation and human cancer. After the positional cloning of PTEN, it was immediately obvious from its sequence that it was a phosphatase (Li et al., 1997; Steck et al., 1997). While it possessed protein tyrosine phosphatase activity (Li et al., 1997), it was subsequently shown that PTEN was also a lipid phosphatase capable of specifically removing the 3′ phosphate from PI(3,4,5)P3 (Maehama and Dixon, 1998). Further

analysis of PTEN mutations showed that this activity was essential to its function as a tumor suppressor (reviewed by Vazquez and Sellers, 2000). Thus, PTEN seems to be designed as a brake for the Class 1 PI3Ks. Not surprisingly, PI3K signaling was found to be highly activated in PTEN-null tumor cell lines and primary tumors. Overall, PTEN is inactivated in a high percentage of common human cancer types including tumors of the prostate (30–50%) (Cairns et al., 1997), brain (at least 30%) (Chiariello et al., 1998) and breast (more than 20%) (Feilotter et al., 1999).

While the discovery of the lipid phosphatase activity of PTEN had heightened interest in PI3K as a potential drug target in cancer, the importance of this class of enzymes in cancer was fixed with the discovery that the PIK3CA gene encoding p110α was mutated at a high frequency in a number of the most common forms of cancer, including colon, breast, prostate, liver, and brain tumors (Samuels and Velculescu, 2004). Studies in several model systems have showed that the mutations commonly found in p110α in human tumors are indeed activating (Isakoff et al., 2005; Kang et al., 2005; Samuels et al., 2005; Zhao et al., 2005). Notably, mutations have not been found in the genes encoding the other Class 1 PI3Ks, even though several groups have demonstrated that these enzymes are capable of acting as oncogenes in model systems (Kang et al., 2005; Zhao et al., 2005). However the PIK3CB gene, encoding p110β, has been found to be amplified in several tumor types.

As drug companies prepare to attack the pathway, an examination of what we have learned from genetic mouse models and chemical genetics might contribute to designing optimal inhibitors for PI3K. Traditional knock-out of p110δ or knock-in of an inactive form of p110δ yields mice that grow to adulthood but show severely impaired T and B cell function (Jou et al., 2002; Okkenhaug et al., 2002). Genetic ablation of either p110α or p110β gives an early embryonic lethal phenotype (Bi et al., 2002; Bi et al., 1999), limiting the information that can be gained about the roles of these isoforms in adults. However, while heterozygous knock-out of either p110α or p110β had no effect on insulin signaling, mice with heterozygous loss of both isoforms showed a noticeably impaired insulin response (Brachmann et al., 2005). Recent studies have used either conditional knock-out of p110α (Zhao et al., 2006) or heterozygous knock-in of a kinase dead allele of p110α (the homozygous knock-in is, as expected, early embryonic lethal) (Foukas et al., 2006) to show that p110α is essential for most RTK signaling and for normal insulin function in animals. When p110β was examined again via knock-in of a kinase dead allele or via conditional knock-out, a very different picture was seen. Unlike p110α, loss of p110β has little or no effect on RTK signaling as usually measured (Jia et al., 2008). Instead p110β is essential for GPCR (G protein coupled receptor) signaling (Jia et al., 2008, Guillermet-Guibert et al., 2008). Surprisingly, p110β also seems to play a role in insulin action in vivo. Even more surprising is the fact that the knock-in allele is viable (E. Hirsch, in press), suggesting that p110b has a kinase independent function. Studies in mouse embryo fibroblasts (MEFs) using a conditional allele support this idea and suggest that the kinase independent function might play a role in membrane trafficking (Jia et al., 2008).

MT antigen is once again an important part of the PI3K story as the search begins to find which p110 isoforms are important to tumor formation. Mice with floxed alleles of p110s are now being bred into a variety of mouse models for cancer. Early results are in for MT and a number of receptor tyrosine kinases in standard MEF transformation system (Utermark et al., 2007). Here it has been found that ablating p110α alone is sufficient to slow, or in the case of MT, completely block tumor growth. Notably these p110 knock-outs have finally allowed confirmation that PI3K is actually essential to MT transformation (Utermark et al., 2007), a nontrivial question since the SH2 domains of other signaling molecules bind sequences similar to the motif at Y315. Other studies are being carried out in genetic mouse models where surprisingly knock-out of p110α has been found to have little effect in a PTEN driven prostate tumor model while ablation of p110β has been found to greatly diminish tumorigenesis in the anterior lobe (Jia et al., 2008). Clearly more studies will be required to find which isoform is the better target in a given tumor type.

The pharma industry has not overlooked the potential importance of PI3K as a drug target in cancer. A large number of companies are now moving aggressively to test PI3K inhibitors in the clinic. Since most if not all of the first inhibitors in the clinic will block all p110 isoforms (and perhaps other kinases related to the PI3Ks as well), some target-directed side effects may be encountered. These might include impaired immune function due to loss of p110δ function as well as problems in insulin response due to inhibition of p110α. However, none of the known side effects of a pan inhibitor need limit its potential therapeutic usefulness, especially if such an inhibitor can be used for a relatively limited course of treatment (perhaps weeks or months instead of the years of treatment employed for some protein kinase inhibitors).

$PLC_{\gamma 1}$ binding to tyrosine 322

The role of tyrosine 322 in PLCγ binding was first recognized by Su et al. (1995), who made their initial observations based on the similarity of the YLDI motif at tyr322 with the then known preferred binding peptides for the SH2 domains of PLCγ. While expression of MT in murine fibroblasts clearly results in enhanced tyrosine phosphorylation of PLCγ, it has been difficult to see MT mediated activation of signaling pathways downstream from PLCγ including activation of PKCs or enhanced calcium release. Mutation of Y322 to F resulted in reduced focus formation when the assay was performed in low serum but not in normal serum. However, expression of MT in the Jurkat T cell line did result in a clear amplification of stimuli, which normally activate calcium signaling and NFAT. This enhanced calcium signaling was dependent on Y322 (Kennedy et al., 1998). In mice, regulated expression of a dominant negative allele of PLCγ markedly decreased lung metastases in the MMTV-MT breast tumor model without altering the primary tumors (Shepard et al., 2007).

Other MT binding proteins

TAZ

MT binds the WW domain of TAZ through amino acid residues 2–4 (Tian et al., 2004). First reported as a 14-3-3 binding protein, TAZ functions in transcriptional regulation (Hong et al., 2005) and also can promote protein degradation through a $SCF^{\beta\text{-}Trcp}$ E3 ligase complex (Tian et al., 2007). TAZ modulates mesenchymal stem cell differentiation (Hong et al., 2005), promotes cell proliferation (Lei et al., 2008) and functions in tumorigenesis of breast cancer cells (Chan et al., 2008). Unpublished reports indicate that a polyomavirus mutant defective in TAZ binding) is unable to transform and induce tumors (Tian et al., 2007). However, such a mutant would also be defective in ST– and LT–TAZ interactions, so how much of the effect is from MT is not certain.

Heat shock 70 family proteins

Most of the first exons (79 amino acids) common to MT, LT and ST comprise a classic DnaJ domain. As such, these proteins would be expected to bind and activate the hsp70/hsc70 family of DnaK cellular heat shock proteins. Infection with either SV40 or polyoma induces the expression of the heat shock proteins (Khandjian and Turler, 1983). For LT, hsc70 binding is known to happen and to be important for activation of E2F-containing promoters (Sheng et al., 1997). While MT might be expected to bind to hsp/hsc70, it appears that PP2A binding and Hsc binding are largely mutually exclusive, with PP2A found bound to the majority of both ST and MT when the viral proteins are expressed at levels found in transformed cells. At high levels of expression of MT, as might be seen upon expression via an adenovirus

vector (Pallas et al., 1989), or when PP2A binding is abrogated by mutation (Campbell et al., 1995; Walter et al., 1987), hsp/hsc70 proteins can indeed be immunoprecipitated with the viral protein. As noted above, the DnaJ domain is important for the recruitment of PP2A and src. However, this does not appear to result from binding and activation of DnaK proteins. Deletion analysis has shown that removal of a key loop with the amino acid sequence HPDK has no effect on the ability of MT to transform (Campbell et al., 1995; Glenn and Eckhart, 1995).

14-3-3 proteins

MT binds 14-3-3 proteins (Pallas et al., 1994) through an interaction at phosphoserine 257 (Cullere et al., 1998). The interaction with 14-3-3 provides an illustration of the importance of tissue context. While not important at most sites, the interaction appears critical for the induction of salivary gland tumors by polyoma virus and important for the generation of fibrosarcomas (Cullere et al., 1998).

The proline-rich region

The C-terminal region of MT contains a proline rich region from residue 332–347 (Fig. 1). The mutant dl1015 that has a deletion in this region (339–347) is defective in transformation (Magnusson and Berg, 1979; Magnusson et al., 1981). This mutant has a normal tyrosine kinase activity associated with it (Schaffhausen and Benjamin, 1981c). Deletion of the three core prolines (residues 336–338) yields a MT defective in inducing tumors in animals (Yi and Freund, 1998). The basis for the defect in transformation is unclear. Identification of a point mutant E349K that mimics the defect (Schaffhausen and Denis, unpublished) suggests PI3 kinase signaling is interrupted between recruiting the enzyme and its downstream signaling.

Finally, it should be noted that several laboratories are pursuing proteomic approaches that are revealing additional MT binding proteins, such as lipin 1 and 2 (Schaffhausen et al. unpublished).

The MT-membrane connection

MT is associated with membranes (Andrews et al., 1993; Ballmer-Hofer and Benjamin, 1985; Dilworth et al., 1986; Ito, 1979; Ito et al., 1977; Schaffhausen et al., 1982; Silver et al., 1978; Zhu et al., 1998). The only portion of the MT sequence long enough and hydrophobic enough to span a lipid bilayer is very close to the C-terminus (Fig. 3). Mutation that renders MT cytoplasmic by truncating prior to the hydrophobic stretch (Py1387T) abolishes its ability to transform (Carmichael et al., 1982). Truncation of 6 amino acids into the C-terminal side of the hydrophobic stretch renders MT non-transforming (Novak and Griffin, 1981).

There is agreement that MT can be associated with a variety of cell membranes. However, the apparent distribution of MT depends on the way that the experiment has been done. Immunofluorescence and immunoelectron microscopy experiments have emphasized association with intracellular membranes, particularly the endoplasmic reticulum (Dilworth et al., 1986; Templeton et al., 1984; Zhu et al., 1984), while biochemical separations have focused more on association with plasma membranes (Ito, 1979; Schaffhausen et al., 1982). In either case, most of the MT molecule is oriented towards the inside of the cell. The orientation of the hexapeptide C-terminus is not yet clear. In either case, MT appears not only to be in contact with membranes, but also with membrane skeleton/cytoskeletal elements (Andrews et al., 1993; Krauzewicz et al., 1994; Schaffhausen et al., 1982). This sublocalization is likely to be important for function, because MT/PTK complexes are enriched in these fractions (Schaffhausen et al., 1982). Elliott et al. (1998) have provided evidence suggesting that the basic cluster N-terminal to the hydrophobic cluster is important for this localization.

The original examination of the membrane anchoring sequence for MT suggested that it might spontaneously associate with membranes in a manner analogous to cytochrome b5. However, in vitro model membrane binding experiments (Kim et al., 1997) suggest that MT carboxy terminal sequences do not spontaneously insert into membrane as does cytochrome b5, but are able to associate electrostatically. The same result is obtained when the anchor sequence is placed on another protein, implying that the rest of the MT sequence is not inhibiting. These results may well suggest that association with one or another MT partner is responsible for allowing its insertion into the bilayer.

The transmembrane domain is not uniquely required for MT transformation. Replacement of the hydrophobic sequence with the C-terminal lipid modification sequence from H-Ras (a CaaX box) restored transforming activity (Elliott et al., 1998). However, the role of the membrane localization sequence is not entirely non-specific either. Substitution of VSV-G protein sequences (Templeton et al., 1984) or cytochrome b5 (Kim et al., 1997) sequences to target MT to membranes did not allow MT transformation. Similarly substitutions within the hydrophobic domain rendered MT non-transforming (Markland et al., 1986). A truncation mutant that leaves basic cluster on MT, while lacking the hydrophobic stretch, is still associated with particulate fractions including cytoskeletal fractions (Elliott et al., 1998). In any case, those insertion sequence mutants are intriguing because they activate src to some extent, but are nonetheless non-transforming (Markland et al., 1986).

Genetic analyses of the regions adjacent to the hydrophobic stretch have also been performed. The hexapeptide (KRSRHF) C-terminal to the hydrophobic sequence can be deleted without affecting transformation of F111 rat embryo fibroblasts (Dahl et al., 1992). However, the N-terminal basic region appears more important. As noted above, that region has been suggested to be important for connection to the cytoskeleton (Elliott et al., 1998). E392G is cold sensitive for transformation (Templeton and Eckhart, 1984a). Mutation R393E just N-terminal to the hydrophobic sequence yields a mutant that is less completely localized to membranes than wild type (Dahl et al., 1992). This mutant has only a weak focus forming activity and is unable to promote anchorage independent growth (Dahl et al., 1992). Both src (65%) and PI3 kinase (85%) activities are affected by the mutation. Other substitutions (K, H, or M) at 393 had no effect.

Animal models of transformation driven by MT

No account of MT mediated transformation would be complete without mention of the many murine tumor models dependent on MT. Polyomavirus induces tumors in numerous tissues in the mouse (Dawe et al., 1987). A great advantage of the system is that signal transduction questions can be tested by tumor studies in animals. The most direct method is by infecting with polyomavirus defective in MT (Bronson et al., 1997; Cullere et al., 1998; Freund et al., 1992a; Freund et al., 1992c; Yi and Freund, 1998; Yi et al., 1997). Not only do hr-t mutants defective in MT and ST show defects in tumor formation (Benjamin, 1982), but 1387T that encodes a cytoplasmic MT mutant, but is wild type for LT and ST, is highly defective in the ability to make tumors. A very important conclusion from all of these studies is that mutations affecting particular signaling pathways have different effects in different tissues. Mutation of the PI3 kinase binding site resulted in a 20-fold reduction in kidney tumors, for example, but there was actually an increase in the number of bone tumors in the mutant as

AHSM^{384}QRHLRRLGR^{393}**TLLLVTFLAALLGICLMLFILI**KRSRHF^{421}

Fig. 3. The sequence at the C-terminus of MT is responsible for association with membranes. The hydrophobic sequence is underlined. Residue 393 that is discussed in the text and residue 384 that is the last residue found in non-transforming, cytoplasmic Py1387T are indicated.

compared to wild type (Freund et al., 1992a). As described earlier, Y250 seems to be critical in many transformation assays in vitro. However, its contribution to the virus' tumor profile is much less dramatic than that of 315 (Bronson et al., 1997; Yi et al., 1997). While the frequency of kidney tumors was dramatically reduced, tumor frequencies in the mammary gland or hair follicles were not much affected. However, the hair follicle tumors caused by the mutant were much larger than those from wild type (Bronson et al., 1997). The interaction of MT with the 14-3-3 proteins appears critical for the induction of salivary gland tumors and important for the generation of fibrosarcomas, but is not important for tumorigenesis at most sites (Cullere et al., 1998).

To test MT in the context of virus requires that the mutant virus replicates in animals. Even if the virus replication is normal, MT signaling is still being tested in the context of LT and ST action on the host. To test the role of MT directly, transgenic approaches have been used. As a transgene MT causes tumors in a wide variety of tissues (Aguzzi et al., 1990; Bautch et al., 1987; Cecena et al., 2006; Guy et al., 1992; Rassoulzadegan et al., 1990; Tehranian et al., 1996; Williams et al., 1988). Some of these models have been discussed briefly above but others deserve mention. In prostate for instance MT can induce fatal prostate cancer (Tehranian et al., 1996). Expressed in endothelial cells, MT induces hemangiomas. These tumors are especially interesting because the transformed endothelial cells recruit non-transformed endothelial cells into the tumors (Primo et al., 2000; Williams et al., 1989). When expressed in mammary glands under control of the MMTV promoter, MT efficiently induces metastatic carcinomas (Guy et al., 1992). This much-used model will be discussed further below. An obvious problem with a traditional transgenic approach to studying tumor development is the time at which the oncogenic signal is generated. One approach to getting around this problem is to use conditional expression of Cre (Cecena et al., 2006) or some kind of tetracycline regulation. An alternative would be to introduce MT via a retrovirus. Historically an avian retrovirus carrying MT was used to induce hemangiomas in chickens (Kornbluth et al., 1986). The current methodology introduces MT into specific tissues using the RCAS-TVA system developed by Varmus and colleagues (Fisher et al., 1999). Mice have been derived that express the ALSV-A (TVA) avian retrovirus receptors in specific tissues. When these mice are infected with RCAS (Replication Competent ALV LTR with a Splice acceptor) viruses, only cells expressing the receptor are infected. For MT, mammary tumors (Du et al., 2006), liver tumors (Lewis et al., 2005) and pancreatic tumors (Lewis et al., 2003) have been examined in this way. The approach has now been extended to lentivirus constructs to allow efficient infection of non-dividing cells (Siwko et al., 2008).

MT transgenic systems have been extensively studied to understand the process of tumorigenesis. The MMTV-MT mammary tumor system originally developed by the Muller lab (Guy et al., 1992) has been particularly widely studied (see Marcotte and Muller, 2008 for a review). It provides a relatively good model for the human disease (Lin et al., 2003; Namba et al., 2004), even though, as noted by Marcotte and Muller there are significant differences from the human situation. The mouse tumors are largely ERα negative, while 50% of human tumors are positive, and the mouse tumors metastasize to the lung, whereas human metastases are more broadly distributed. In the MMTV-MT 634 line, by 5 weeks of age all mice had palpable tumors that involved the whole mammary fat pad; 94% of females developed metastatic lung disease by 3 months of age (Guy et al., 1992). Genetic analysis indicates that src, but not yes, is important for the development of MT tumors (Guy et al., 1994). Both the activation of PI3K and Shc is thought to be important for MT tumorigenesis (Webster et al., 1998). Tumor formation defects in a MT mutant 250 can be ameliorated by overexpression of either Shc or Grb2 (Rauh et al., 1999). Akt is an important downstream target of PI3 kinase. Ablation of Akt 1 inhibits, but ablation of Akt2 enhances, mammary tumor formation by MT (Maroulakou et al., 2007). The experiments of Webster described above also removed 322 from MT, but the role of binding to PLCγ has not been directly tested by mutation of only the 322 site. However, the expression of dominant negative $Plc_{\gamma 1}$ reduced metastasis, suggesting that 322 might play a role in that process (Shepard et al., 2007). Induction of metastasis seems to depend on the production of osteopontin (Jessen et al., 2004), which is regulated by MT at the transcriptional level through both Shc and PI3K (Whalen et al., 2008). A remaining question is whether MT is functioning as "one-hit" tumor inducer, or whether MT initiates a series of events that lead to other genetic changes required for tumor formation. The high frequency and short latency might seem to argue that MT is all that is needed. On the other hand, there is obviously progression of the lesions (Lin et al., 2003), and it is possible to isolate mammary intraepithelial neoplasias that have different properties (Maglione et al., 2004) and show different patterns of expression (Namba et al., 2004, 2006). A difficulty in the interpretation of such experiments is that it has long been known that the transformed phenotype varies with the amount of T antigen expression (Raptis et al., 1985). Little is known about the expression levels of MT protein in different lesions.

Studies on animals point out a variety of additional issues that need to be considered when thinking about MT signaling. It is quite obvious that the strain of mice being used makes a significant difference. For polyomavirus, there are large differences in susceptibility to tumor formation (Freund et al., 1992b) that depend to a significant extent on differences in the nature of the immune response (Lukacher et al., 1993, 1995; Velupillai et al., 1999, 2002). In the transgenic models there are also striking differences in latency and metastatic potential when MT is expressed in different mouse strains (Lifsted et al., 1998; Winter and Hunter, 2008). A bioinformatics approach has pointed to c-myc and cdc25a as genes regulating latency (Cozma et al., 2002). Sipa1, which is a Rap GAP has been implicated as a modifier of metastasis (Park et al., 2005). Finally, the interaction of MT cells with their neighbors will affect the signaling patterns. As noted above, MT transformed endothelial cells recruit non-transformed endothelial cells (Primo et al., 2000; Williams et al., 1989). Adipocytes, one of the cell types of the stroma in mammary tissue, can produce collagen VI that affects primary MT tumor growth (Iyengar et al., 2005). Macrophages, for example, seem to contribute to both progression and metastasis in the MT-MMTV system (Lin and Pollard, 2004). There appears to be a signaling loop between tumor cells and macrophages that is involved in tumor cell migration (Wyckoff et al., 2004).

Epilogue

By this point it should be apparent that the study of MT has contributed much to our knowledge of signaling and transformation. What must also be underscored is how much remains to be done. Not only do we need to understand known binding proteins better, but new proteins are being discovered by more modern and sensitive proteomic technologies. The genetics of MT suggest that there are important aspects of the signaling in transformation that are not yet understood. The ability of MT transgenes to drive tumor formation in so many tissues and the vast store of mouse lines combining MT with other genetic lesions should enable MT transgenics to be excellent models to decipher tissue and tumor specific roles of signaling molecules, such as p110α/β, going forward. Another important challenge is translating the tissue and mouse strain specific responses that are known to occur into a molecular understanding of the regulation of and by MT.

Acknowledgments

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