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Review

MYC-y mice: From tumour initiation to therapeutic targeting of endogenous MYC

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ABSTRACT

MYC is one of the best-studied oncogenes in terms of mouse models of malignancy. MYC overexpression has been targeted to several tissues using transgenic constructs, and more recently as mouse models have evolved, conditional systems have been developed to allow the regulation of MYC expression or activity *in vivo*. The ability to target MYC expression to specific tissues and cell lineages, as well as the ability to regulate that expression, has made genetically engineered mouse models (GEMM) a valuable resource for studying the importance of MYC in the process of tumourigenesis. Here we review how these models have been used to address the role of MYC in tumour initiation and maintenance, how subtle changes in levels of MYC can influence tumourigenesis, and finally the ongoing efforts to target endogenous MYC genetically and with novel therapies.

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1. Introduction

c-MYC is an oncogene frequently overexpressed in human tumours (Marcu et al., 1992) and as such has been the subject of a great deal of research. As one of the first oncogenes to be identified, *MYC* has been the subject of investigation for over 30 years. *MYC* was initially identified as a retroviral oncogene in avian tumours (Duesberg et al., 1977), and subsequent research showed that, in common with many oncogenes identified in retrovirally transformed cells, there was a cellular homologue of this gene, referred to as *c-MYC* (Sheiness et al., 1980; Vennstrom et al., 1982). Later studies revealed that this oncogene belongs to a family of genes that includes *c-MYC*, *L-MYC*, *MYCN*, *B-MYC* and *s-MYC* (Ingvarsson et al., 1988; Nau et al., 1985; Slamon et al., 1986; Sugiyama et al., 1989; Vennstrom et al., 1982), however, of these only *c-MYC*, *L-MYC* and *MYCN* have been associated with malignancy (DePinho et al., 1991; Nesbit et al., 1999).

A role for MYC in driving human cancer was first identified in Burkitt's lymphoma, where *MYC* was deregulated as a result of chromosomal translocation into the immunoglobulin heavy chain locus (Dalla-Favera et al., 1982; Neel et al., 1982; Taub et al., 1982). Since then, the *c-MYC* proto-oncogene has been shown to be deregulated in a wide range of human and animal malignancies (Dang, 1999; Spencer and Groudine, 1991), and is estimated to be overexpressed in over 50% of all human tumours.

Although identified as an oncogene, it became clear that *c-MYC* was vital for normal development, and for regulation of cell proliferation. As a nuclear transcription factor, MYC regulates several hundred target genes involved in regulating cell growth and proliferation, differentiation, apoptosis,

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metabolism, angiogenesis and DNA repair (Dang, 1999, 2012). MYC is unable to bind DNA alone however, and requires oligomerisation with a binding partner to bind DNA effectively (Kato et al., 1992). An HLH/LZ protein called MAX was identified as a binding partner for c-MYC in humans (Blackwood and Eisenman, 1991), and the murine homologue of MAX, known as MYN, was shown to have the same function (Prendergast et al., 1991). Initially it was expected that MYC-MAX heterodimers bound to E box motifs in target DNA to activate transcription, and that MAX–MAX homodimers repressed the activity of MYC by competing with MYC-MAX heterodimers for DNA binding sites on target genes (Kato et al., 1992). This theory was complicated somewhat by the discovery of a third family of proteins, the MAD proteins, which are also able to bind MAX (Ayer et al., 1993). MAD-MAX heterodimers are also able to repress the transcriptional activity of MYC.

The potency of *c-MYC* as an oncogene relies upon the fact that MYC is a crucial regulator of proliferation, through its ability to regulate the cell cycle. Regulation of the cell cycle by c-MYC has been attributed to its ability to transcribe a number of genes involved in cell cycle progression, for example the cyclins and the cyclin dependent kinases (Barrett et al., 1995; Daksis et al., 1994; Jansen-Durr et al., 1993; Perez-Roger et al., 1997; Rudolph et al., 1996). There is now a significant body of evidence that c-MYC regulation of the cell cycle can occur at multiple levels. In common with other potentially oncogenic proteins, however, c-MYC can also activate apoptosis, and as the transactivation and DNA binding domains are required for apoptosis it is thought that c-MYC affects the transcription of certain genes involved in apoptosis (Evan et al., 1992; Stone et al., 1987). More recent research has suggested that the mechanism by which c-MYC induces apoptosis is more complex than originally supposed, and that rather than having the ability to directly mediate cell death as a result of all these triggers, c-MYC can sensitise cells to a wide range of apoptotic stimuli by causing release of cytochrome *c* into the cytoplasm, the consequence of which will be dependent on other cellular signals (Juin et al., 1999).

2. Modelling Myc deregulation in the mouse

The importance of MYC for development was first uncovered by attempts to generate *Myc* knockout mice. Mice nullizygous for *c-Myc* failed to survive past embryonic day 9.5 (Davis et al., 1993), while *Mycn* deletion was lethal at embryonic day 10.5. Thus, the ability to target gene expression to specific tissues and cell lineages in transgenic mice, as well as the ability to regulate that expression, has made genetically engineered mouse models (GEMM) a valuable resource for studying oncogenic function, and the importance of MYC in the process of tumourigenesis. Expression of the putative oncogene can be activated in tissues of interest, at the appropriate time, and the resulting changes in phenotype used to understand the actions of that gene. Oncomice have been important not only for advancing the understanding of oncogenic activity *in vivo*, but have also resulted in the generation of well characterized cell lines expressing particular oncogenes, for use in mechanistic studies *in vitro*. In addition, collaborating tumourigenic events have been identified in transgenic mice already harbouring a constitutively activated oncogene, and synergy between different oncogenes can be examined in this way.

The *c-MYC* oncogene is among the best-studied transgenic models of malignancy, and c-MYC overexpression has been targeted to particular tissues using transgenic constructs with different regulatory elements. More recently, as mouse models have evolved, conditional systems have been developed to allow the regulation of myc expression or activity *in vivo*. One such strategy has used transgenic constructs of *Myc*, fused to the tamoxifen inducible oestradiol receptor, such that MYC can be activated post-transcriptionally by induction with tamoxifen. Other models have used tetracycline-based systems to conditionally activate or inactivate transcription of *Myc* in a spatio-temporal manner. In these models, *Myc* is coupled to a Tet-O promoter and *Myc* expression is then regulated by the activity of a second, tissue-specific transgene that encodes either the tet-transactivating protein (tTA) or the reverse tet-transactivating protein (rtTA). When mice are fed doxycycline, binding of the tTA to Tet-O is blocked and *Myc* expression suppressed (Tet-off), while in the converse Tet-on system, *Myc* expression is switched on by doxycycline-induced binding of the rtTA to Tet-O.

These models have been used very successfully to study the effects of *Myc* deregulation in adult mice, thus removing any concerns over the effects of aberrant activation or inhibition of *Myc* during development. The role played by *Myc* in driving tumorigenesis in a variety of different tissues has been studied in this way, and a role for *Myc* as a 'driver' mutation in many different malignancies has been proposed as a result of these studies. Some of these studies are summarized in Table 1, and we will discuss them further below. In addition, the dependence on MYC for tumour maintenance has been studied in these models, and we will discuss insights from these models and the implications for anti-myc therapies in human cancer.

3. The role of MYC in tumour initiation

Although *MYC* is mutated in many human tumours, the contribution of *MYC* mutations is difficult to ascertain given the late stage at which tumours are diagnosed and the number of genetic lesions present. Because of this, mouse models of *Myc* deregulation have been invaluable in establishing to role of *Myc* in tumour initiation.

One of the earliest models of MYC-induced tumourigenesis was described in 1985 by Adams et al. (Adams et al., 1985). In this model, *c-Myc* was fused to an immunoglobulin enhancer ($E\mu$) in an effort to recapitulate the translocations frequently observed in human lymphomas. This approach proved fruitful, as mice developed both pre-B cell and mature B cell lymphomas within a few months from birth, demonstrating that *Myc* overexpression alone could drive lymphomagenesis. In fact, further studies indicated that deregulation of *c-Myc* may be a fundamental event in the development of lymphoid neoplasia. Proviral integration at *c-Myc* was observed in almost 50% of murine leukaemia virus induced T cell

Table 1 – Evidence for a role for *Myc* as a 'driver' mutation in various tissues from several mouse models.

Model	Tissue	Outcome	Reference
Eμ-c-Myc	Haematopoietic	Pre-B cell lymphoma Mature B cell lymphoma	Adams et al., 1985
CD2-c-Myc	T cell lineage	T cell lymphoma	Stewart et al., 1993
MMTV-c-Myc	Mammary	Mammary adenocarcinoma	Stewart et al., 1984
Probasin-c-Myc	Prostate	PIN, invasive adenocarcinoma	Ellwood-Yen et al., 2003
Tyrosine Hydroxylase-MYCN	Neuroectodermal	Neuroblastoma	Weiss et al., 1997
SP-C-c-Myc	Lung alveolar epithelium	Multifocal bronchio-alveolar hyperplasia, adenomas, carcinoma	Ehrhardt et al., 2001
UG-c-MYC	Lung clara cells	T cell lymphoma, polycystic kidneys, renal cell carcinoma, Clara cell hyperplasia of the lung	Geick et al., 2001
Ela-tv-a, Cdkn2a-/- + RCAS-c-Myc	Pancreas	Pancreatic endocrine tumours	Lewis et al., 2003

lymphomas (Selten et al., 1984), while in a model of T cell lymphoma in which a transgenic construct of *c-Myc* is placed under the control of a *CD2* locus control region promoter, *Myc* overexpression was sufficient to induce thymic lymphoma in a subset of mice (Stewart et al., 1993). Thus, the potency of *Myc* as a major driving force behind haemopoietic malignancies was been demonstrated in these mice.

The potential for *Myc* deregulation to initiate tumours has also been studied in many epithelial cancers. For example, when *Myc* was fused to the LTR of the mouse mammary tumour virus (MMTV) to generate a model of *Myc* overexpression in mammary tissue, mice developed mammary tumours, but typically in only one or two mammary glands and only in multiparous females (Stewart et al., 1984). These results suggested that MYC was not sufficient to drive mammary tumourigenesis, and that further mutations were required for transformation. In contrast, in a model of prostate cancer, mice harbouring *c-Myc* under the control of a probasin promoter all developed prostatic intraepithelial neoplasia that progressed to invasive adenocarcinoma. Tumours in these animals were highly reminiscent of a subset of human, 'Myc-like' human cancers, in phenotype and genetics, reinforcing the value of these mice in modelling the human disease. (Ellwood-Yen et al., 2003). In addition these results highlight the utility of animal models in distinguishing driver mutations from 'passenger' mutations.

We should not forget that *MYCN* is frequently deregulated in human cancer, and in particular, is often amplified in human neuroblastomas. The ability of *MYCN* amplification to drive neuroblastoma was therefore tested in mice engineered to overexpress *Mycn* in neuroectodermal cells. These mice did indeed develop neuroblastoma with many of the molecular features of the human disease, providing the first proof that *MYCN* may be a driver of human neuroblastoma (Weiss et al., 1997).

Targeting expression of one oncogene to different cell types of the same tissue can also provide clues to the likely cells of origin of cancer in certain tissues. For example, in a lung tumour model in which *c-Myc* is expressed under the control of a surfactant protein C (SP-C) promoter, mice developed multifocal bronchio-alveolar adenomas that progressed to adenocarcinoma but with long latency and incomplete penetrance (Ehrhardt et al., 2001). When a Clara Cell promoter was used to drive aberrant *c-Myc* expression, however, only bronchioloalveolar hyperplasia was observed (Geick et al., 2001), suggesting that deregulated myc expression is not sufficient to drive tumourigenesis in these cells.

In contrast, studying the role of *Myc* in tumour models has also provided insights into how genetic events can influence the genesis of different tumour types from the *same* target cells. Lewis and colleagues created a model using an avian retroviral receptor under the control of the elastase promoter to deliver oncogenes in viral vectors to the pancreas. When *c-Myc* expression was targeted to elastase expressing cells, the resulting tumours were exclusively pancreatic endocrine tumours, while delivery of mouse polyoma virus middle T antigen (PyMT) resulted in the formation of either acinar or ductal pancreatic tumours. Thus, targeting expression of oncogenes to a specific subset of cells can provide understanding of the outcome of oncogene activity in potentially multipotent progenitor cells (Lewis et al., 2003).

4. Lessons from co-operating genetic events

Because cancer is a multi-stage process requiring a series of genetic events, mouse models of *Myc*-driven tumours have also been used to identify co-operating mutations that can accelerate tumourigenesis, thus providing insight into the molecular pathways that normally prevent cells with deregulated myc from transformation.

Models in which *Myc* deregulation can drive tumour initiation have been studied for many years in an effort to identify co-operating genetic events that enhance the tumourigenicity of MYC and thus to better understand the mechanisms of MYC-induced tumourigenesis. In some early experiments, the laboratories of Anton Berns and Jerry Adams performed insertional mutagenesis screens in the *Eμ-Myc* model to identify cooperating oncogenes and tumour suppressor genes. This system identified of a number of important regulatory genes, including *Pim-1* and *Bmi-1* (Haupt et al., 1991; van Lohuizen et al., 1991), two genes involved in tumourigenesis but also in stem cell homeostasis. Further, in *Eμ-Myc* mice, acceleration has been observed following introduction of *Pim-1* (Verbeek et al., 1991), as well as *Ras* (Langdon et al., 1989) and the *Bcl2* oncogene (Vaux et al., 1988). *Myc* transgenic models have also been crossed with mice lacking tumour suppressor genes, and in particular, there has been great interest

in *Myc*-induced tumourigenesis on a *Trp53* deficient background. The co-operation between *c-Myc* and *Bcl-2* in tumourigenesis (Strasser et al., 1990), adds weight to the theory that loss of apoptotic function may be important for the full oncogenic potency of *c-MYC*.

Some investigators have suggested a requirement for wild-type p53 in c-MYC induced apoptosis (Hermeking and Eick, 1994; Wagner et al., 1994), while studies from other groups however, have reported p53 independent c-MYC induced apoptosis (Blyth et al., 2000; Hsu et al., 1995; Sakamuro et al., 1995). Many studies have shown that overexpression of *Myc* and loss of p53 co-operate in tumourigenesis (Blyth et al., 1995; Elson et al., 1995; Hsu et al., 1995). *CD2-Myc* mice null for *Trp53* for example, develop thymic lymphoma at an increased incidence and decreased latency, compared to *Trp53* heterozygous littermates (Blyth et al., 1995), while in *Eμ-Myc* animals, overexpression of *c-Myc* could cooperate with heterozygous deletion of *Trp53* or its regulator $p19^{ARF}$ in B cell lymphomagenesis (Schmitt et al., 1999). Investigation of the levels of apoptosis in tumours arising in these models however, indicated that loss of p53, or loss of heterozygosity in $Trp53^{+/-}$ tumours, did not inhibit the ability of MYC to induced apoptosis (Blyth et al., 2000; Hsu et al., 1995). These results suggested that in these tumours MYC did not require p53 to induce apoptosis, and rather, loss of *Trp53* could collaborate with overexpression of *MYC*, because of enhanced cell cycle progression and proliferation in the absence of p53.

Similarly, the Fas:FasL death receptor pathway had been implicated in MYC-induced apoptosis in some cell types (Hueber, 1997), and when *Eμ-Mycl* were crossed onto a Fas deficient background they showed an accelerated rate of tumour formation of both B and T-cell origin (Zornig et al., 1995). However, a separate study indicated that the apoptosis mediated by c-MYC was independent of the Fas signalling pathway (Yeh et al., 1998), and in the *CD2-Myc* model of T cell lymphoma, tumour development and levels of apoptosis were unchanged by the absence of Fas (Cameron et al., 2000).

Other model systems have been used effectively to identify genetic events that can cooperate with *Myc* to drive tumourigenesis, thereby shedding light on the mechanisms employed by MYC to drive tumourigenesis. For example, transgenic expression of *Myc* in pancreatic islet cells is not sufficient to induce tumours, but rather causes rapid apoptosis, a loss of beta cells, and the subsequent onset of diabetes. When this apoptosis is suppressed by co-expression of a *Bcl-xL* transgene, c-MYC is able to drive proliferation resulting in the formation of aggressive islet cell tumours (Pelengaris et al., 2002). Over the years, studies of cooperation between *Myc* and other genes in a number of tumour types have been invaluable in identifying the mechanisms by which MYC can drive tumourigenesis, and also those that prevent transformation of MYC-overexpressing cells.

5. The role of Myc in tumour maintenance

Perhaps most importantly, the regulatable mouse model systems that have been developed over the past 15 years have enabled researchers to study the outcomes of inactivation of MYC activity in established tumours in a variety of different tissues. A number of models have now provided evidence that *Myc* is not only a driver of cancer in many tissues, but that a number of *Myc*-driven tumours are 'addicted' to myc expression since inactivation of *Myc*, at least initially, leads to tumour regression in many cases. We have summarised these findings in Table 2.

The first study to address the dependence of *Myc*-driven tumours on continued *Myc* expression was performed in a haematopoietic model using the tet-off system to regulate *Myc* expression (Felsher and Bishop, 1999). Prolonged *Myc* expression resulted in the development of both T cell lymphomas and acute myeloid leukaemias. When *Myc* was then 'switched off', these tumours spontaneously regressed, and this regression was associated with proliferative arrest, differentiation and apoptosis of the tumour cells (Felsher and Bishop, 1999). These data were supported by a later study using the tet-off system to regulate *Myc* in lymphoid cells (Marinkovic et al., 2004).

In time, a number of groups reported similar findings in a number of different types of cancer. Using the *MycER*™ transgene targeted to the epidermis, Pelengaris and colleagues showed that continued activation of MYC resulted in the formation of papilloma-like lesions. When MYC activity was then suppressed, even transiently, through administration of tamoxifen, the papillomas regressed completely and the tumour cells underwent growth arrest and an irreversible differentiation (Pelengaris et al., 1999). A similar outcome was observed in bone, using the tetracycline regulatory system to inactivate *Myc* in model osteosarcomas. Transient inactivation in this model resulted in tumour regression and differentiation of sarcoma cells into mature bone. Again this differentiation appeared irreversible, as reactivation of *Myc* was insufficient for tumour regrowth but rather induced apoptosis in the targeted cells (Jain et al., 2002).

Research into the consequences of *Myc* inactivation in *Myc*-driven mammary tumours yielded somewhat contrasting results and highlighted the importance of cancer stem cells, at least in this tumour type. The Chodosh laboratory demonstrated that tetracycline regulated *Myc* overexpression was able to drive the formation of invasive mammary adenocarcinomas. Following *Myc* withdrawal, however, most of these tumours continued to grow, and even in those tumours that did regress, some cells did remain which could rapidly reform a tumour following *Myc* reactivation, or even spontaneously (Boxer et al., 2004). When the group investigated this apparent progression towards *Myc*-independent tumour growth, they found that Ras was very frequently mutated in the tumours that could continue to grow in the absence of *Myc*, but not in the tumours that fully regressed (D'Cruz et al., 2001). Their results offered evidence of a hard-to-kill subset of tumour cells that can remain dormant but are able to drive recovery of the tumour following *Myc* reactivation. Experiments in model systems like these will be very useful in modelling disease progression, but also mechanisms of resistance to therapy, and may offer clues as to why some treatments are only effective in subsets of patients in the clinic.

Researchers using models of *Myc*-induced tumourigenesis in other organs to investigate the phenomenon of 'oncogene addiction' have reported similar findings. In pancreatic islet

Table 2 – Evidence that a number of tumours in *Myc*-driven mouse tumour models are 'addicted' to MYC.

Model	Organ	Myc on	Myc off	Reference
EμSR-tTA x tet-O-MYC	Haematopoietic	T cell lymphoma, acute myeloid leukaemia	Complete regression, proliferative arrest, apoptosis, irreversible differentiation	Felsher and Bishop, 1999.
Eμ-tTA x tet-O-MYC	Haematopoietic	Invasive T- or B-cell lymphoma	Complete regression, apoptosis, differentiation	Marinkovic et al., 2004
Involucrin-c-MycER™	Skin epidermis	Papilloma	Complete regression, recovery of normal skin	Pelengaris et al., 1999
EμSR-tTA x tet-O-MYC transplanted osteogenic sarcoma cells/primary transgenic tumours	Immature osteoblasts	Invasive and metastatic osteogenic sarcoma	Complete and sustained regression, differentiation to mature bone, Insensitive to MYC reactivation	Jain et al., 2002
MMTV-rtTA x tet-O-MYC	Mammary	Mammary adenocarcinoma	Continued tumour growth in ~50% of cases, full regression in ~50%	D'Cruz et al., 2001
MMTV-rtTA x tet-O-MYC	Mammary	Mammary adenocarcinoma	Continued tumour growth. Spontaneous recurrence in few tumours that regress.	Boxer et al., 2004
pIns-c-MycER™	Pancreas	Invasive pancreatic β cell tumours	β cell apoptosis, rapid regression. Rapid recurrence following MYC reactivation	Pelengaris et al., 2002 Pelengaris et al., 2004
Glt1-tTA x tet-O-MYCN/luc	Brain	Medulloblastoma	Rapid senescence, clearance of apoptotic cells. Residual senescent tumour cells evident after 80 days	Swartling et al., 2010
LAP-tTA x tet-O-MYC	Liver	Hepatocellular carcinoma, rapidly in neonates, long latency in adults	Sustained regression, differentiation. Tumour regrowth from dormant cells on MYC reactivation	Beer et al., 2004, Shachaf et al., 2004

cells, activation of MycER™ in combination with expression of Bcl-xL triggered rapid tumourigenesis (Pelengaris et al., 2002). Inactivation of MYC did lead to regression, however reactivation of MYC in this context led to rapid tumour regrowth (Pelengaris et al., 2004). Thus, the genetic profile of tumours, as well as the cells in which they arise, may affect the consequences of oncogene de-activation. This might be addressed by studies in a GEMM of medulloblastoma in which mice bearing tetracycline responsive *MYCN* were crossed to mice expressing tTA under the control of the glutamate transporter 1 (Glt1) promoter (Swartling et al., 2010). These mice developed medulloblastoma displaying classic or large cell anaplastic (LCA) pathology with rare metastases. When *MYCN* was deactivated in mice with established tumours, tumour cells rapidly entered senescence and previously apoptotic cells were cleared. Senescent cells remaining in the regressed tumours were still evident 80 days after *MYCN* deactivation. The finding of genomic instability in tumours in this model suggests the acquisition of additional mutations is required for progression of MYC-driven tumours, and thus that MYC is necessary, but not sufficient to drive tumourigenesis. One might predict that upon MYC reactivation, tumour recurrence might be observed in these mice. As such, this represents an interesting model in which to test whether *in vivo* senescence is truly irreversible.

Finally, *Myc* regression models have provided insights into the consequences of oncogene expression and withdrawal at different developmental stages. For example, when *Myc* was overexpressed in the hepatocytes of embryonic or neonatal mice, proliferation leading to neoplasia resulted occurred very rapidly. However when *Myc* was activated in the hepatocytes of adult mice, there was a lack of proliferation, and instead cell growth was induced, with tumours only developing with long latency. Although cancer is generally thought to be associated with ageing, these data suggested that adult cells may acquire mechanisms, lacking in the cells of immature animals, to prevent tumourigenic responses to activation of any on oncogene (Beer et al., 2004). In tumours in a similar model, *Myc* inactivation led to a robust tumour regression, with tumours cells differentiating into hepatocytes and biliary cells, however in contrast to the findings in haematopoietic, skin and bone tumours, dormant cells remained that could quickly re-establish tumours upon reactivation of *Myc* (Shachaf et al., 2004).

Clearly, the specific effects of *Myc* inactivation depend on the tumour type, the role played by *Myc* in the initiation of the tumour, and likely the genomic instability of the tumour. One can envisage a situation in which the more unstable the genetics of the tumour, the more likely that tumour may be able to become *Myc*-independent in the event of *Myc* inactivation. *Myc* inactivation in tumour models has also provided insights into the types of cells from which tumours originate. Given the transcriptional targets of MYC, it is not surprising that cell cycle arrest and apoptosis are among the consequences of *Myc* inactivation in some tumours. However, differentiation and the conversion of tumour cells to apparently normal tissue in some cases of *Myc* withdrawal and the subsequent outgrowth of tumours when *Myc* is restored support the existence of cancer stem cells. In contrast, in other tumour types, tumour regression is sustained, following withdrawal of *Myc*. The level of genomic instability, and spontaneous mutations arising during the process of *Myc*-induced tumourigenesis

very likely shapes the consequences of disengagement of *Myc*.

6. Low-level MYC overexpression in murine cancer models

Most of the work discussed so far has examined mouse models where MYC is exogenously expressed at very high levels to initiate tumourigenesis (either alone or when combined with other oncogenic or tumour suppressor mutations). In human cancer, apart from perhaps Burkitt's lymphoma and neuroblastoma, this scenario is quite unlikely. Indeed a number of studies have shown MYC levels to progressively increase during human carcinogenesis by multiple mechanisms, so exogenous high expression of MYC alone might not be the best way to model the role of MYC in human malignancy (reviewed by (Myant and Sansom, 2011b)). Moreover, the regression studies discussed above may produce very different results if the tumours had not been driven by MYC overexpression and instead by other mutations that are common in human cancer. One of the first studies to examine the consequences of the levels of MYC overexpression used *Myc-ER*^{TM} cDNA targeted into the *Rosa26* locus (which is expressed at equivalent levels to endogenous c-MYC but lacks either 5′ and 3′ regulatory control)(Murphy et al., 2008). This low level MYC overexpression led to a very distinct phenotype from the high levels of overexpression previously performed as the apoptotic pathways often engaged by MYC overexpression were not activated in most tissues and thus increased proliferation was seen in most organs. The only place apoptosis was observed was in the colon where the *Rosa26* locus is most highly expressed and this could be suppressed by the co-deletion of p19^{ARF}. Interestingly, these mice were relatively resistant to tumourigenesis compared with previous MYC overexpressing mice with only lung tumours formed at long latencies. Future studies crossing these mice to mice that model additional tumour promoting events will show whether this small increase in MYC can promote tumour progression. Another recent study showing that threshold levels of MYC are important for initiating tumourigenesis again targeted *Myc* into the *Rosa26* locus. In contrast to the ER approach, this study also placed *Myc* under the control of cre recombinase, as a *LSL* (lox stop lox) cassette was placed before the *Myc* cDNA (Wang et al., 2011). This study also investigated the impact on post-translation control of MYC stability. Previously MYC has been shown to be regulated by phosphorylation of T58 and S62. Here the T62 phosphorylation is an activating phosphorylation which stabilises MYC but is followed by phosphorylation at T58 which triggers dephosphorylation of T62 thus targeting MYC for degradation. Therefore this study targeted a T58A *Myc* mutation, which should prevent degradation, to the *Rosa26* locus and compared this to wild type *Myc*. It should be noted that mutations at T58 have been observed in human cancer. Interestingly, in contrast to overexpressing wild type *Myc* in this context, the T58A allele led to mammary cancer when crossed to the *Wap-Cre*. The requirement for the precise amount of MYC was highlighted again, by the finding that 2 copies of *Myc*^{T58A} were needed at the *Rosa26* locus to drive cancer.

7. Endogenous MYC overexpression in murine cancer models

The *Myc* oncogene encodes one of the most highly regulated proteins in the cell. It is a target of many key transcriptional pathways and is also controlled post-transcriptionally with a rapid turnover. In human cancer many different mechanisms have been shown to deregulate MYC activity. Of all cancers, colorectal cancer (CRC) has been identified as one where MYC is deregulated by multiple control mechanisms all of which should lead to the progressive accumulation of MYC and this has been verified as functionally important in murine models of cancer. In CRC, the *APC* (Adenomatous Polyposis Coli) gene is mutated in approximately 80% of tumours (Kinzler et al., 1991; Kinzler and Vogelstein, 1996). APC is a negative regulator of the WNT signalling pathway, and loss leads to the accumulation of β-catenin in the nucleus and the activation of TCF/LEF (or WNT) target genes (Bienz and Clevers, 2000). c-MYC is a target of WNT pathway with TCF4 binding sites its promoter (He et al., 1998). Definitive proof of the significance of endogenous MYC was provided by studies deleting *c-Myc* following *Apc* gene deletion in the murine intestine. Deletion of *Apc* within the mouse intestine yields a crypt progenitor cell like phenotype with intestinal enterocytes failing to differentiate, continuing to proliferate and failing to migrate (Sansom et al., 2004). Associated with this hyperproliferation is a relocalisation of β-catenin to the nucleus and the activation of WNT target gene expression, with an increase of *Myc* RNA of about 4-fold. To assess the significance of MYC overexpression, *Myc* was then co-deleted with *Apc* and found to rescue all of the phenotypes of *Apc* gene deletion (Sansom et al., 2007). Interestingly, the majority of WNT target genes were also downregulated by the deletion of *Myc* suggesting that within the intestinal epithelium MYC is required for the full expression of the WNT programme. This would fit well with recent studies suggesting MYC as an amplifier of transcriptional programmes (Lin et al., 2012; Nie et al., 2012). One caveat with this study is that all of MYC was deleted and not just WNT-inducible MYC, however other murine studies have tried to address this important point. First, in mice that carry a single *Apc* mutation and go on to develop polyps, *Myc* heterozygosity has been shown to slow tumourigenesis and tumour progression (Athineos and Sansom, 2010; Buchert et al., 2010). When both copies of *Apc* are deleted the crypt progenitor phenotype is also reduced by *Myc* heterozygosity despite a 2-fold increase in Myc compared with wild type intestines. Moreover, in addition to this crypt progenitor phenotype being induced by WNT/MYC signalling it has been shown that during intestinal regeneration there is an increase in both β-catenin and Myc when the intestines regrow. Once again in this scenario MYC was required for the ability of the intestine to regenerate. Importantly, when *Myc* was expressed at the *Rosa26* locus using the LSL approach whilst endogenous *Myc* was deleted, yielding wild type levels of MYC but not WNT-inducible, the gut was unable to regenerate suggesting that it is the induction of MYC in this case that is require for intestinal regeneration (Ashton et al., 2010). In support of this finding, a recent study examined the importance of a 3′ WNT responsive element in the *Myc* promoter. Germline

deletion of this element led to a two-fold increase in MYC expression in the colon of knockout mice, and resulted in increased proliferation and more efficient regeneration of the colon following injury caused by DSS-induced ulcerative colitis (Konsavage et al., 2012).

Underlining the importance of MYC to colorectal cancer was the finding that there one of the most important susceptibility loci in colorectal cancer mapped to 8q24 (the *Myc* enhancer). Initially there was some controversy whether this modified expression of *Myc* (Pomerantz et al., 2009; Tuupanen et al., 2009). However a recent elegant study which highlights the power of GEMM to definitively answer these functional questions deleted this cancer associated single nucleotide polymorphism (SNP) from mice (Myc-355 mice). Interesting whilst mice were overtly normal and only had a small reduction in MYC levels, when they were crossed to the Apc^{Min} mouse tumourigenesis was markedly suppressed (Sur et al., 2012).

In addition to these transcriptional control mechanisms, many post-transcriptional mechanisms that reduce MYC activity are also altered in colorectal cancer. Mutations in *KRAS* and *FBW7* (the latter which degrades MYC) are frequent during colorectal cancer and accelerate tumourigenesis in the mouse (Sancho et al., 2010; Sansom et al., 2006). Other proteins that control MYC stability, such as HectH9, USP28 and CIP2A, are also deregulated which could all lead to further MYC activity (Adhikary et al., 2005; Junttila et al., 2007; Popov et al., 2007). Moreover mir34b/c, which binds to the 3 UTR in *MYC* and targets the message for degradation, is downregulated in a large percentage of colorectal cancer either epigenetically or by inhibition of mechanisms that control its expression (Cannell et al., 2010; Kress et al., 2011; Myant and Sansom, 2011a). Further studies altering these proteins in GEMM models of intestinal cancer should define their functional significance *in vivo* and gauge whether they might act as therapeutic targets in colorectal cancer.

One other question that remains is the tissue specific nature of MYC activity downstream of WNT signalling and the mouse also allows modelling of this in different systems. Deletion of *Apc* within the liver leads to hyperproliferation and the accumulation of c-MYC (Reed et al., 2008). However in contrast to β-catenin deletion, MYC deletion in these mice has no impact and livers still hyperproliferate. This might be relevant to human HCC as those liver cancers that have a poor prognosis often have an activation of β-catenin but also amplification of MYC, suggesting that within the liver the Myc levels driven by β-catenin alone are not sufficient for transformation. Consistent with this, MYC was not required for the expression of Wnt targets genes within the liver (in contrast to the intestine).

8. Targeting endogenous MYC

The data from CRC as well other cancers therefore suggests that targeting endogenous MYC function might be an excellent therapeutic approach for cancer. Two major concerns remain, first, will be there be a therapeutic window since normal cells do require c-MYC in development and there are longer term phenotypes in the intestine and skin when *Myc* is deleted (Muncan et al., 2006). Second, what percentage of MYC will need to be inhibited within a tumour to cause regression? Thus far, most studies modulating endogenous c-MYC had done so from tumour onset and not in an established tumour. Recent studies using mouse models to target endogenous MYC function in tumours have begun to address this.

The most promising data that MYC ablation in established tumours can drive regression has come from using a transgene where a dominant negative MYC protein is expressed under the control of tetracycline. This "*OmoMyc*" transgene was crossed to a *CMV-rtTA* mouse, which then meant that in mice given doxycycline in the drinking water this transgene would be expressed throughout tumour-bearing mice (Soucek et al., 2008). This should then inhibit Myc function in both normal and tumour tissue addressing both questions of therapeutic window and regression of established tumours. A number of questions remain over the precise mechanism of action of this *OmoMyc* transgene which binds to the DNA binding domain of MYC and should stop MYC:MAX dimers (but not MYC:MIZ dimers) but could have a number of off target effects that have at the moment not been addressed. The results with the *OmoMyc* transgenic mice have been remarkable. Inhibition of MYC function in established *Kras*-driven adenomas of the lung caused a complete regression (Soucek et al., 2008). Within normal tissues such as skin and gut there was reduced proliferation but when the transgene was switched off (by removal of doxycycline from the drinking water) these tissues now returned to normal homeostasis. This effect of *OmoMyc* is not simply restricted to the lung as *OmoMyc* expression also causes regression of RIP-TAG induced pancreatic islet tumours (Sodir et al., 2011). In this case, when the kinetics of regression was examined, a dramatic collapse of tumour vasculature was observed first which was then proceeded by death of the tumour. Thus, this was the first evidence that Myc inhibition might not only affect tumour cells but also the surrounding stromal cells.

9. Drugging MYC or downstream pathways

The conclusions from mouse models are therefore pretty clear: MYC is an excellent therapeutic target for cancer. There is a therapeutic window, disease indication and tumour regression occurs when inhibiting both exogenous and endogenous MYC. However MYC as a transcription factor is very difficult to target and up until recently there have been no compounds that worked in mouse models *in vivo* that could target MYC. This has somewhat changed recently, given the discovery that in some circumstances inhibitors to the Bromodomain protein BRD4 can inhibit MYC activity. Use of BRD4 inhibitors showed inhibition of a signature of genes which are upregulated in *Eμ-Myc* mice, and in xenografts of malignant myeloma, acute myeloid leukaemia and Burkitt's Lymphoma showed both a reduction in MYC based transcription and tumourigenesis (Delmore et al., 2011; Mertz et al., 2011). Although it is unlikely that MYC is the only target of BRD4, and there may again be cell type specificity, this raises the exciting prospect of compounds that target MYC activity within the clinic in the near future. Moreover, the suite of mouse models discussed in this review should rapidly

elucidate whether BRD4 inhibition works in multiple settings where cells are dependent on MYC, and should drive the clinical development of this set of compounds.

Given the difficulty in designing inhibitors to MYC, other approaches have been made to try and target downstream pathway members where inhibitors are currently available. Following *Apc* loss, for example, there is a MYC dependent activation of both Cyclin D2 and FAK and deletion of both of these slows *Apc* loss-induced tumourigenesis (Ashton et al., 2010; Cole et al., 2010). Here it would be interesting to combine CDK4 and FAK inhibitors to see if this could, in part, mimic MYC inhibition. A recent study by the Downward laboratory acts as an excellent paradigm of this (Kumar et al., 2012). Here, looking for agents that could specifically kill *KRAS* mutant cells, they found that knockdown of GATA2 was synthetically lethal with *KRAS* mutation. Although as a transcription factor this was difficult to target, they identified 3 major pathways downstream of GATA2 that were important for the viability of *KRAS* mutant cells and where inhibitors were available. They then treated murine KRAS-driven lung tumours and showed that treatment with the combination of inhibitors caused tumour regression in this GEMM model. Similar studies are underway working on MYC, and exciting results have shown that inhibition of CHK1, ARK5 and SUMOylation (Kessler et al., 2012; Liu et al., 2012; Murga et al., 2011) all preferentially kill cells expressing high levels of c-MYC. Future studies with inhibitors and genetic inhibition of these proteins in the MYC dependent mouse models will validate these targets in an *in vivo* setting. This is vital given the number of synthetic lethal studies in tissue culture which have not transferred into more robust and clean genetic systems *in vivo*.

It over 25 years since the first transgenic model that showed that MYC can drive tumourigenesis. These models have shaped our understanding of *MYC* as an oncogene and shown that even in established tumours MYC is required to maintain transformation. AS GEMM models have become more complex and more closely recapitulate human cancer, the precise role of MYC in the tumourigenic process has been established and its important function and its key transcriptional targets have been revealed. Given the recent excitement that MYC may be targetable in cancer this means that we already have an amazing set of tools to test whether 1) these inhibitors elicit a therapeutic response, 2) whether they work through MYC, and 3) whether they are tumour type specific. It would be a mistake if drug companies ignored these elegant models in the rush to be the first-in-class to be licenced, as these models should provide stratification and efficacy. Moreover, this set of mouse models may also provide important information about resistance mechanisms. This was elegantly shown in a mouse model of PI3 Kinase driven breast cancer where treatment with inhibitors to this pathway regressed tumours but recurrence was driven by an overexpression (via amplification) of MYC or MET (Liu et al., 2011).

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