

Importance of MUC1 and spontaneous mouse tumor models for understanding the immunobiology of human adenocarcinomas

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Abstract Many important aspects of cancer biology, such as cancer initiation, progression, and metastasis, have been studied in animal models, mostly mice. As long as cancer was considered primarily a genetic disease, the study of transplantable mouse tumors, or even human tumor xenografts in immunocompromised mice, appeared to suffice. Many important genetic events that lead to transformation and in vivo tumor growth were elucidated. However, many even more important factors that determine whether or not the genetic potential of a tumor cell will be realized, such as the host response to the tumor and the tumor microenvironment that influences this response over a long period of time of tumor development, remained untested and unappreciated. This is slowly changing with the advent of molecular techniques that have spurred efforts to engineer better mouse models of human tumors. In this review, we show results of our efforts to combine a genetic mouse model of spontaneous human adenocarcinomas based on a Kras mutation, with an important human molecule MUC1 that is abnormally expressed on human adenocarcinomas, promoting oncogenesis, proinflammatory tumor microenvironment, and immunosurveillance.

Keywords Lung cancer · Pancreatic cancer · Cancer vaccine · Tumor-associated antigens · Mucin

Introduction

Genetically engineered mouse models intended to recapitulate many aspects of human carcinogenesis have become available in the last 5–10 years. Among them are mice that harbor a latent mutation in the codon 12 (G→D) of one of the two Kras alleles, which when activated in a tissue of choice gives rise to a tumor of that tissue [1]. Many human tumors carry Kras mutation and some, like pancreatic cancer, lung cancer, and colon cancer, in large percentages. Thus, many aspects of the development of these cancers, from the initial mutation through progression from premalignant to fully malignant lesions, can be

studied using this model. Many papers have already been published using the KrasG12D mice in combination with other genetically engineered strains to study human tumors. There is no doubt that some tumor cell intrinsic processes applicable to human tumors have been revealed by these studies. As can be expected, however, there are nevertheless many features of human tumors that cannot be recapitulated due to numerous differences between mice and man, suggesting the need for additional genetic manipulations.

One molecule that has been shown to be important in the process of oncogenesis in human adenocarcinomas, in determining the nature of their microenvironment and in their immunosurveillance, is the glycoprotein MUC1 [2]. Human MUC1, and the distant mouse homologue Muc1, have only limited structural and functional similarities but are mostly profoundly different in their function. It is thus very likely that the absence of human MUC1 in most mouse models that have been used to study human epithelial adenocarcinomas, and in particular in the KrasG12D

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mouse model of spontaneous tumors, can lead to erroneous conclusions about the mechanism of human oncogenesis, the kinetics of human tumor growth and progression, the nature of the pro-tumor versus anti-tumor microenvironment, and the outcome of spontaneous tumor immunosurveillance, which are all shown to be in some way influenced by MUC1 on human tumors.

MUC1 promotes tumorigenesis

MUC1 is the first described and best-known member of the mucin family that currently has 20 members (reviewed in ref. [2]). This family is characterized by transmembrane-associated and secreted glycoproteins, which show a dense O-glycosylation linked to serine-, threonine-, and proline-rich tandem repeat domain called VNTR (variable number of tandem repeats). The VNTR domain of MUC1 is comprised of 20–120 tandemly repeated 20 amino acid segments whose sequence includes five potential O-glycosylation sites per repeat. In its normal expression, MUC1 is found at low amounts on the apical cell surface of most glandular epithelial cells. There, it performs a variety of biological functions, such as lubrication and hydration of epithelia and protection against colonization by pathogens. It is also involved in cell–cell (via E-cadherin) and cell–matrix (via integrins) interactions as a part of both adhesion and anti-adhesion mechanisms. Adhesion effects are mediated by carbohydrate structures on the extracellular repeat domain, whereas anti-adhesive effects can be mediated by the glycosylation-stabilized rigid conformation of the several hundred-nanometer-long mucin molecules. Its highly conserved cytoplasmic domain can be phosphorylated at seven phosphorylation sites by, among other signaling proteins, glycogen synthase kinase 3 β , c-src, and protein kinase C δ and thus regulate further cell signaling, through interaction with β -catenin. MUC1 furthermore associates with members of the ErbB family of receptor tyrosine kinases and FGFR3 [3–7].

During tumorigenesis, cells lose their polarity and MUC1 expression is found at very high levels on the entire cell surface. The extracellular tandem repeat region can act as a ligand for intercellular adhesion molecule (ICAM) 1 involved in metastatic extravasation. The O-glycosylated extended ectodomain can receive signals from selectin binding or conceivably trigger signaling events. Overexpression of MUC1 competes with E-cadherin for binding to Wnt effector β -catenin, modulates the localization of β -catenin to the cytoplasm, and subverts E-cadherin-mediated cell adhesion in epithelial cells, leading to destabilization of intercellular junctions, which benefits tumor cell migration. MUC1 has been shown to bind to the signaling mediators Grb2/SOS upon phosphorylation and to mediate activation of numerous receptor tyrosine kinases.

Activation of the ras signaling pathway phosphorylates Raf, MEK, and ERK1/2, with the latter translocating to the nucleus and inducing transcription of genes involved in mitogenesis, differentiation, apoptosis, and quiescence. Expression of high levels of MUC1 has been shown to elicit EGF-dependent activation of ERK1/2 MAPK. Moreover, MUC1 can mediate resistance to apoptosis in a survival response to oxidative stress by suppression of H₂O₂-induced accumulation of reactive oxygen species (ROS) and in response to DNA-damaging agents, suggesting its important contribution to the resistance of cancer cells to genotoxic agents [8–11].

MUC1 manipulates tumor microenvironment

We [12] and others have shown that tumor-associated forms of MUC1 are chemotactic for circulating immature human myeloid DCs. This is mediated by the peptide epitopes in the tandem repeat region of the underglycosylated MUC1. Moreover, glycopeptides from the same region provide a maturation/activation signal for the DCs that migrate to the tumor site. These DCs that have matured under the influence of MUC1 produce IL-6 and TNF- α , cytokines that have been implicated in tumor metastasis and progression. They also fail to promote a type 1 response. Hence, instead of promoting adaptive immunity for efficient immune surveillance, these DCs promote inflammation that mediates immunosuppression at the tumor site and later increased tumor invasion within the tumor microenvironment. A striking example of the “wrong” immune response that exists in this MUC1-modified tumor microenvironment is induction by the DCs at the tumor site of T cells that secrete IL-13, a cytokine involved in suppression of tumor-specific CTL and direct promotion of growth of tumor cells that bear IL-13 receptor. In addition, it was [13] reported recently that MUC1 regulates p53 transcription by binding to its regulatory domain. P53 is a tumor suppressor gene whose function is often inactivated either through mutation or overexpression in a variety of cancers. Overexpression of MUC1 by tumor cells can lead to aberrant overexpression of p53. MUC1 expression also leads to decreased apoptosis of tumor cells under oxidative stress that occurs naturally during tumor expansion or in response to genotoxic agents. This might be related to the ability of MUC1 to modulate transcription of the nuclear factor κ B (NF- κ B) as well as to regulate the ERK1/2 pathway and AP-1-mediated transcription [14]. The involvement of MUC1 in these networks reveals its key role in regulating tumorigenesis.

The impact of MUC1 on shaping the tumor microenvironment is well illustrated by a study in which MUC1 expression on tumor cells was suppressed by RNA interference. The siRNA-treated pancreatic cancer cell line with

low level of MUC1 expression showed a greatly reduced proliferative and metastatic capacity [15]. High levels of sialyl-Tn antigen, a carbohydrate structure decorating MUC1 on tumor cells, have been linked to increased tumor cell migration in vitro and decreased cell adhesion to the extracellular matrix molecules collagen type I, collagen type IV, and fibronectin. Moreover, MUC1 has been identified as a potential target in the colonization of metastasizing tumor cells by interacting with ICAM-1 or with E-selectin.

MUC1 is expressed on cancer stem cells

The presence of cancer stem cells in tumors has crucial implications for tumor therapy and targeting molecules that are expressed on cancer stem cells or have biological importance in their persistence and tumor recurrence may become of crucial importance. We have recently determined and published that MUC1 is expressed on cells that have all the agreed-upon characteristics of human cancer stem cells [16]. A publication that followed ours also defined a MUC1 isoform that drives the proliferation of stem cells [17].

MUC1 is a target of immune surveillance

In normal epithelia, MUC1 expression is found in small amounts and restricted to the apical cell surface. However, in >80% of most premalignant lesions that are precursors to cancer and other adenocarcinomas, MUC1 expression is highly up-regulated; for instance, in breast cancer, it is expressed up to tenfold higher than in normal cells. MUC1 expressed on tumor cells displays striking alterations with respect to shortened glycan chains (Tn-antigen (GalNAc1-O-S/T) and Thomsen-Friedenreich (TF) or T antigen (Galβ1-3GalNAcα1-O-S/T)), increased sialylation (mono- and di-sialylated TF-antigen, mono-sialyl Tn-antigen, sialyl-core1), and a shift in the carbohydrate core-type. This is important for the humoral and cellular immune response, because the peptide core is more accessible for peptide-specific anti-MUC1 antibodies and for enzymatic processing into peptides for presentation to T cells, compared with MUC1 on normal epithelia (reviewed in [2]).

Based on these well-documented functions of human MUC1, most of which are not shared by the mouse homologue, many observations about the biology of tumor development and the host response to the tumor made in mouse tumor models in the absence of this important human molecule we believe will need to be re-evaluated in new models that incorporate human MUC1. Below, we review our efforts to establish such models and our preliminary evidence that the presence of MUC1

modulates the process of tumor development, including its immunobiology.

We have taken advantage of the availability of human MUC1Tg mice [18] that carry the human MUC1 transgene expressed under the endogenous MUC1 promoter and have the correct temporal and spatial pattern of MUC1 expression. In a healthy MUC1Tg mouse, low-level expression of normal, fully glycosylated MUC1 is found on the apical surfaces of ductal epithelial cells of the lung, pancreas, mammary glands, prostate, distal convoluted tubules and collection ducts of the kidney, gall bladder, salivary glands, GI tract, and uterus. Organs that do not normally express MUC1, such as the liver, heart, spleen, and muscle tissue, are negative. Importantly, malignant transformation leads to loss of polarization, overexpression, and underglycosylation of MUC1, fully phenocopying MUC1 expression on human tumors. These mice have been used in our laboratory for many years in studies related to spontaneous and vaccine-elicited MUC1 immunity. Human MUC1, when expressed in the mouse, is capable of engaging in and affecting all the previously described human cell–cell interactions. This is due to the conservation between the two species of specific ligands that MUC1 engages with, such as the mannose receptor, lectin receptors, and pattern recognition receptors.

We have bred human MUC1 transgenic mice with several engineered mouse strains susceptible to tumor development and will describe some of these new mouse models and results obtained with them that reflect the clinical picture of human adenocarcinomas.

KrasG12D/MUC1 mice and spontaneous lung cancer

The Kras spontaneous mouse tumor model was developed by Tyler Jacks and David Tuveson [1] based on the activation of the Kras membrane-associated GTPase signaling protein. Exchanging a glycine for aspartic acid (Kras G12D) at codon 12 results in decreased GTPase exchange activity and thus constitutive signaling through ras signaling pathways. Ras mutations are found in 30% of all human tumors and in the majority of lung and pancreatic adenocarcinomas. Kras mutations cause a cascading pathway of other mutations and cellular dysregulation, including loss of cell cycle control. This mutation is kept silent with the preceding lox-stop-lox sequence that can be removed through the action of Cre recombinase to cause tumorigenesis. The recombinase can be administered either as an exogenous recombinant protein or as a component of a viral vector, or can be expressed endogenously under tissue-specific promoters.

We crossed KrasG12D mice with MUC1 transgenic mice with the expectation that tumors that develop would,

like human tumors, be MUC1 positive. First, we confirmed that MUC1 was expressed normally on the epithelial ducts in the lung. Figure 1 shows a section of a healthy lung from a KrasMUC1 mouse showing appropriate expression of the MUC1 protein only on the ductal epithelial cells polarized to the apical surface facing the lumen of the duct. The antibody used to stain for MUC1 expression is commercially available and specific for an epitope in the tandem repeat region of the extracellular domain of MUC1 that is not modulated by different degrees of glycosylation.

To elicit lung cancer, we administered intranasally commercially available adenovirus carrying Cre recombinase (Adv-Cre) to activate transcription of the mutated KrasG12D allele. At various times post-treatment, we killed mice and examined their lungs for tumor development. Lung sections were analyzed for the presence of premalignant lesions and abnormal expression (overexpression and hypoglycosylation) of MUC1. Histology results showed disorganization of the epithelia and an increase in expression of the tumor form of MUC1 as detected by a specific antibody. Gross lesions were apparent in mice 9 weeks after the administration of Adv-Cre. Lesions exhibited the tumor form of MUC1 (Fig. 2). Compared with the KrasG12D mice, expression of MUC1 in KrasMUC1 mice led to the development of twice as many premalignant and malignant lesions (data not shown). Most of the lesions in the KrasMUC1 mice were characterized by large numbers of infiltrating cells (not shown), the full

characterization of which can provide one of the important clues to the previously observed tumor-promoting function of this molecule.

KrasG12D/MUC1 mice and spontaneous pancreatic cancer

To elicit cancer in the pancreas, we generated either double-transgenic mice Krasp48, by breeding the KrasG12D mice with mice that express Cre recombinase under the pancreas-specific promoter (p48 mice), or triple-transgenic KrasMUC1p48 mice, by breeding the Krasp48 with MUC1Tg. Both Krasp48 and KrasMUC1p48 develop pancreatic tumors over a period of 6–9 months (Fig. 3). The presence of MUC1, like in the case of the lung cancer model above, promotes faster tumor development and more numerous lesions. Figure 4 shows a section of a typical large tumor arising in the KrasMUC1p48 mouse that has many morphological characteristics of a human pancreatic tumor. The highly abnormal looking MUC1⁺ ductal structures are enveloped in prominent tumor stroma. This fibrotic stroma at late stages of cancer development appears to have replaced a significant cellular infiltrate that characterizes early stages of tumor development. Figure 5 shows a fluorescence-activated cell sorter (FACS) analysis of T cells in the healthy pancreas of a healthy MUC1Tg mouse compared with the pancreases of mice developing pancreatic intraepithelial neoplasia (PanIN) lesions. While

Fig. 1 A healthy lung of a KrasMUC1 mouse showing normal MUC1 expression. **a** Tissue section stained with an isotype control antibody; **b** stained with an anti-MUC1 antibody

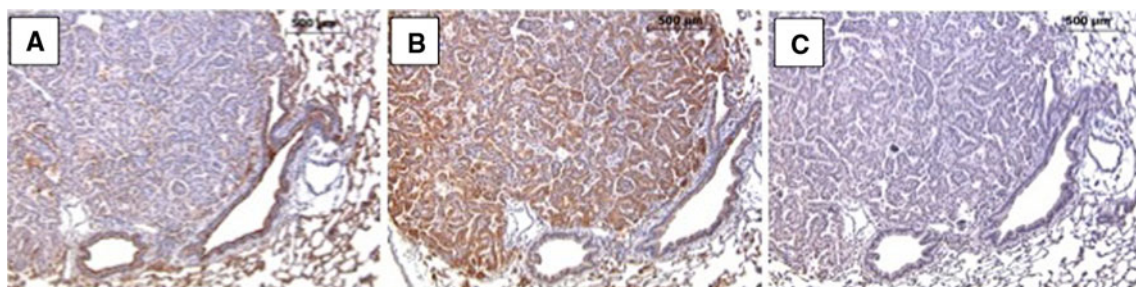
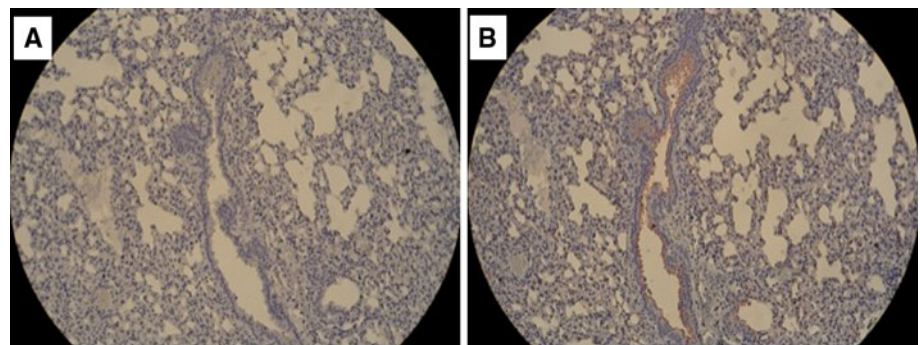
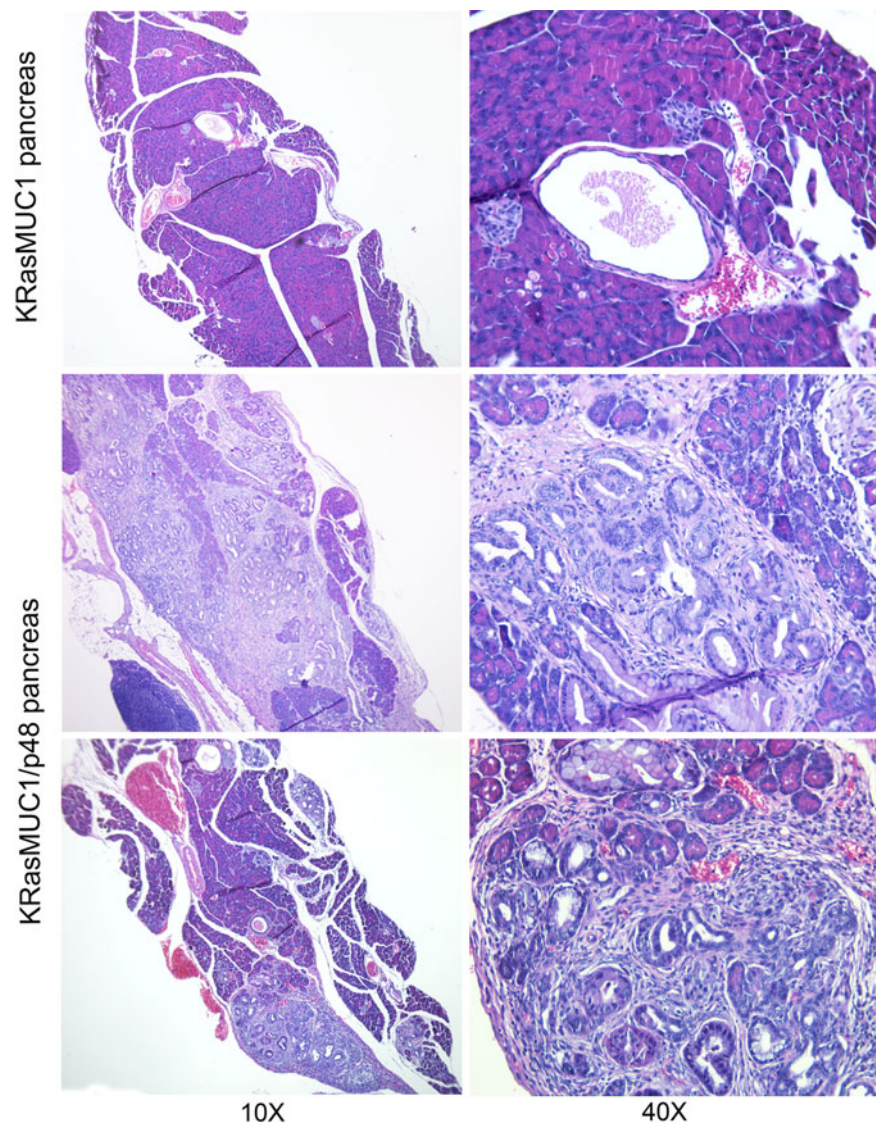


Fig. 2 Lung tumor development and MUC1 expression in KrasMUC1 mice. **a** Staining with an anti-MUC1 antibody 3C6 for normal MUC1 expression; **b** staining with and anti-MUC1 antibody 4H5 for tumor MUC1 expression; **c** staining with an isotype control antibody

Fig. 3 Spontaneous MUC1⁺ pancreatic tumor development. Pancreas in the *top left* (low magnification) and *right* (higher magnification) panels is normal in double-transgenic KrasMUC1 mice. Highly malignant tumors in triple-transgenic KrasMUC1/p48 mice are shown in the *middle* and *bottom left* (low magnification) and *right* (higher magnification) panels



in the healthy pancreas, we detect no T cells; they can be found in pancreases where tumor development was initiated. The difference, however, is striking in the intensity of infiltrates in the absence and in the presence of MUC1 on the PanIN. PanINs negative for MUC1 appear to attract CD4 T cells only and in low numbers. On the other hand, the presence of MUC1 attracts large numbers of both CD4 and CD8 T cells, and they have an activated phenotype as measured by the expression of the activation marker CD69.

Testing a MUC1 vaccine for the prevention of MUC1⁺ tumors in KrasMUC1 mice

In addition to being able to study the important role of MUC1 in the tumor development, having fully immunocompetent mice allows also the study of the host response to the tumor using MUC1 as a tumor antigen. We have

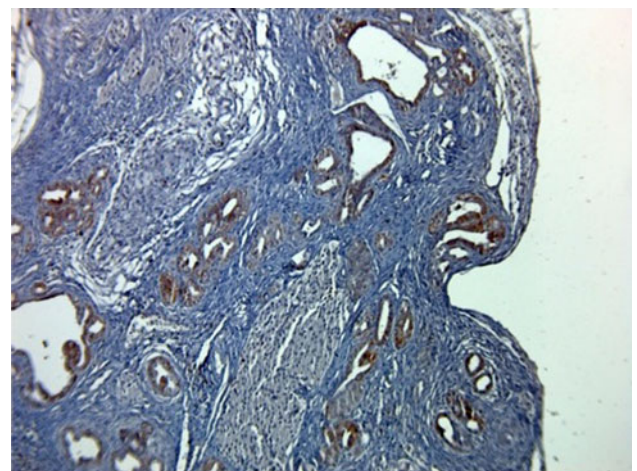
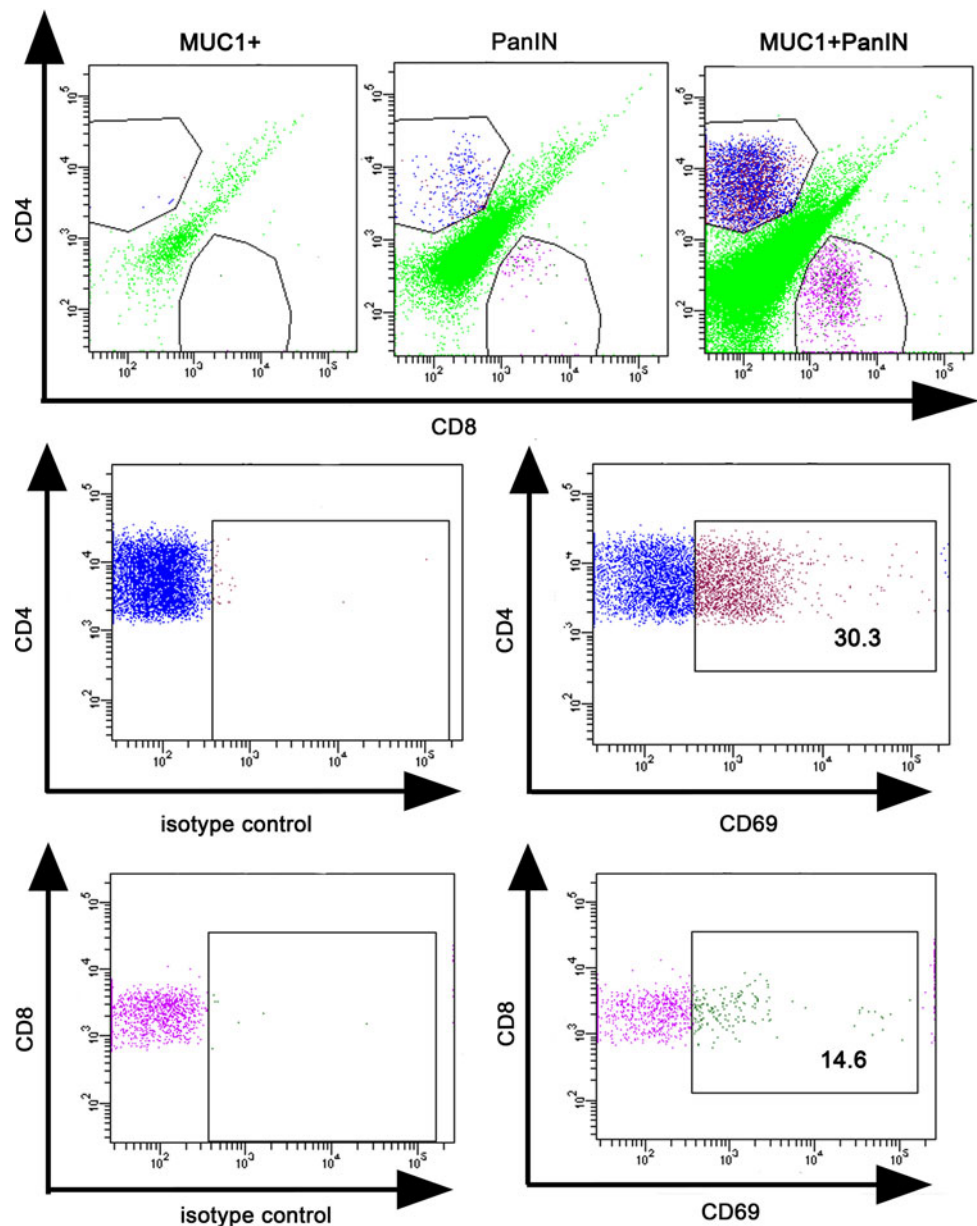


Fig. 4 MUC1 expression on the pancreatic tumor in KrasMUC1/p48 mice. Section stained with antibody 4H5 specific for the tumor form of MUC1

Fig. 5 T cells infiltrating pancreases of mice developing pancreatic intraepithelial neoplasms (PanIN). *Top panels* the highest percentage of CD4 and CD8 T cells are found in response to MUC1⁺ PanINs, and they have activated phenotype (CD69)



started to explore the potential of different MUC1 vaccines to elicit anti-MUC1 immunity in these new mouse models of MUC1⁺ spontaneous tumor development. Most of the work over the last several decades on human tumor antigen-based vaccines and many other forms of immunotherapy directed against human tumor antigens has been done on transplantable mouse tumors usually transfected with individual antigens. Considering the importance of the tumor microenvironment for successful induction of immunity, effective immunosurveillance, and tumor elimination, this approach is no longer valid for most of the questions that are being asked. For the experiment presented in Fig. 6, we initiated lung cancer in 4- to 6-week-old KrasMUC1 mice by intranasal administration of

Adv-Cre described above. At day 7, we started injections of a vaccine composed of 30 μ g of the 100aa-long MUC1 glycopeptide Tn100mer, admixed with 3 μ g of a TLR4-directed adjuvant, described in our recently published study in a spontaneous colon cancer model [19]. Four injections were given at days 7, 21, 36, and 49. Control groups received either adjuvant alone or phosphate-buffered saline (PBS) instead of the vaccine. At day 56, all mice were killed and lungs were prepared for immunohistological analysis for tumor formation and MUC1 expression. Figure 6 shows three mice, one per each treatment group. There is abnormal MUC1 expression as well as evidence of dysplastic ducts and adenoma formation in both PBS (panels A–C)- and adjuvant-only (D–F)-injected mice,

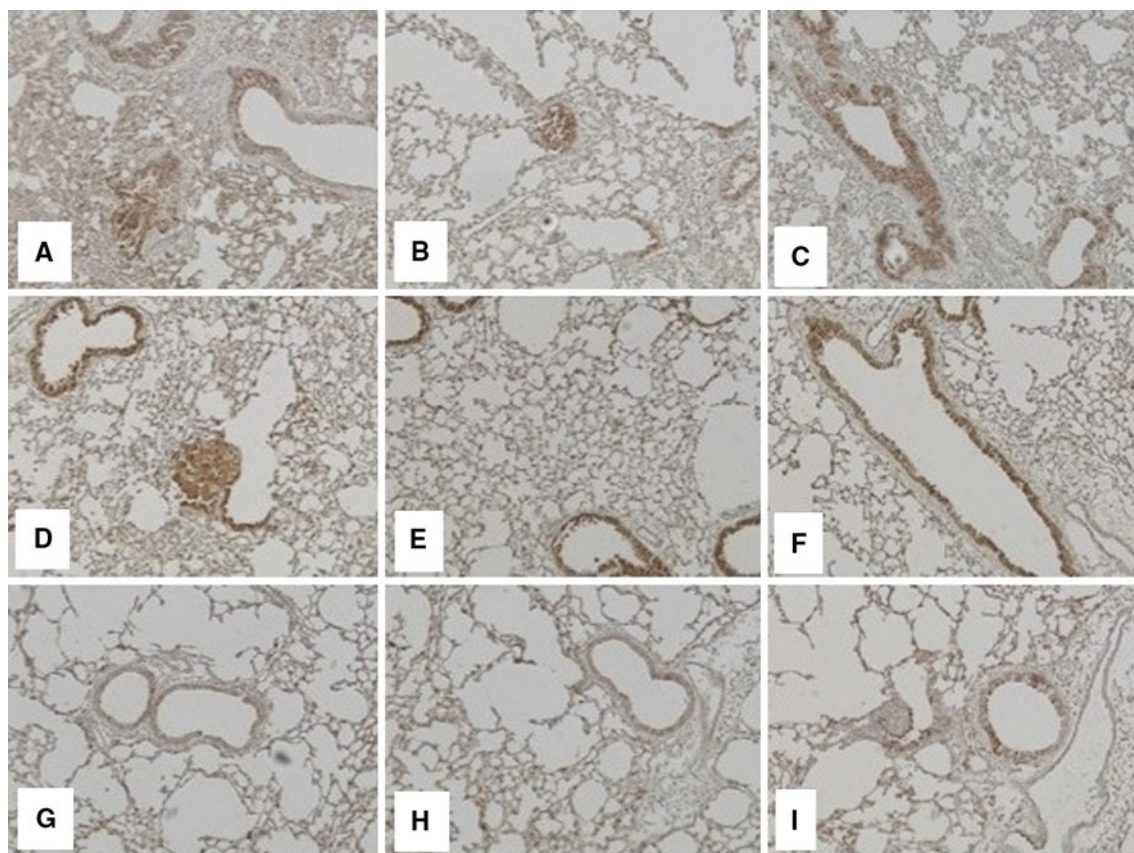


Fig. 6 MUC1-based vaccine for the prevention of MUC1⁺ spontaneous lung tumors in KrasMUC1 mice. **a–c** Three sections of a lung from a PBS-injected mouse; **d–f** three sections of a lung from a mouse

given adjuvant only; **g–i** three sections from a mouse vaccinated with MUC1 plus adjuvant. Sections were stained with the anti-MUC1 antibody 4H5 that recognizes abnormal MUC1

while the vaccinated mouse shows low or no MUC1 expression and normal lung morphology. Similar results were obtained in a prophylactic setting where the vaccine was administered prior to the cancer initiation and boosts given during an early period of cancer development. The vaccine fully protected these highly susceptible mice from developing cancer.

Conclusion

Our one goal here was to illustrate both the importance and the potential of creating and using mouse models that due to technological developments and increasing knowledge of the biology of a specific human disease, are getting ever closer to the human situation. This has become very important for studying human cancer biology and the host response to cancer, where it is now clear that as spontaneous cancer develops, it changes the overall physiology of the host in a way that a short-term growth of a transplantable tumor cannot recapitulate. As a

result, a true picture of processes that are important for tumor progression and therapies that might control them cannot be fully revealed. Our more specific goal was to emphasize the importance of including the human glycoprotein MUC1 into the mouse models, as its importance as an oncogene, an inflammatory signal, and a target for immunosurveillance has been amply supported by work of many groups. The data we have shown here support all of these MUC1 functions by showing that spontaneous mouse tumors that develop in mice engineered to express MUC1 develop faster, attract a larger host cell infiltrate, and are susceptible to immune control. We further show that abnormal MUC1 expression can be targeted by a vaccine, leading to tumor elimination. This positive outcome provides a strong impetus to further study the mechanisms of protection in larger numbers of mice and in additional genetic crosses that eliminate or supply various components of the immune response. These types of studies are impossible to perform with cancer patients and difficult to design for healthy people at risk and thus development and use of appropriate mouse models needs

to remain a priority for basic and translational cancer immunology research.

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References

1. Tuveson DA, Shaw AT, Willis NA, Silver DP, Jackson EL, Chang S, Mercer KL, Grochow R, Hock H, Crowley D, Hingorani SR, Zaks T, King C, Jacobetz MA, Wang L, Bronson RT, Orkin SH, DePinho RA, Jacks T. Endogenous oncogenic K-ras(G12D) stimulates proliferation and widespread neoplastic and developmental defects. *Cancer Cell*. 2004;5:375–87.
2. Vlad AM, Kettel JC, Alajez NM, Carlos CA, Finn OJ. MUC1 immunobiology: from discovery to clinical applications. *Adv Immunol*. 2004;82:249–93.
3. Li Y, Bharti A, Chen D, Gong J, Kufe D. Interaction of glycogen synthase kinase 3beta with the DF3/MUC1 carcinoma-associated antigen and beta-catenin. *Mol Cell Biol*. 1998;18:7216–24.
4. Li Y, Kuwahara H, Ren J, Wen G, Kufe D. The c-Src tyrosine kinase regulates signaling of the human DF3/MUC1 carcinoma-associated antigen with GSK3 beta and beta-catenin. *J Biol Chem*. 2001;276:6061–4.
5. Li Y, Ren J, Yu W, Li Q, Kuwahara H, Yin L, Carraway KL 3rd, Kufe D. The epidermal growth factor receptor regulates interaction of the human DF3/MUC1 carcinoma antigen with c-Src and beta-catenin. *J Biol Chem*. 2001;276:35239–42.
6. Ren J, Li Y, Kufe D. Protein kinase C delta regulates function of the DF3/MUC1 carcinoma antigen in beta-catenin signaling. *J Biol Chem*. 2002;277:17616–22.
7. Ren J, Raina D, Chen W, Li G, Huang L, Kufe D. MUC1 oncoprotein functions in activation of fibroblast growth factor receptor signaling. *Mol Cancer Res*. 2006;4:873–83.
8. Pandey P, Kharbanda S, Kufe D. Association of the DF3/MUC1 breast cancer antigen with Grb2 and the Sos/Ras exchange protein. *Cancer Res*. 1995;55:4000–3.
9. Olayioye MA, Neve RM, Lane HA, Hynes NE. The ErbB signaling network: receptor heterodimerization in development and cancer. *EMBO J*. 2000;19:3159–67.
10. Yin L, Huang L, Kufe D. MUC1 oncoprotein activates the FOXO3a transcription factor in a survival response to oxidative stress. *J Biol Chem*. 2004;279:45721–7.
11. Ren J, Agata N, Chen D, Li Y, Yu WH, Huang L, Raina D, Chen W, Kharbanda S, Kufe D. Human MUC1 carcinoma-associated protein confers resistance to genotoxic anticancer agents. *Cancer Cell*. 2004;5:163–75.
12. Carlos CA, Dong HF, Howard OM, Oppenheim JJ, Hanisch FG, Finn OJ. Human tumor antigen MUC1 is chemotactic for immature dendritic cells and elicits maturation but does not promote Th1 type immunity. *J Immunol*. 2005;175:1628–35.
13. Wei X, Xu H, Kufe D. Human MUC1 oncoprotein regulates p53-responsive gene transcription in the genotoxic stress response. *Cancer Cell*. 2005;7:167–78.
14. Thompson EJ, Shanmugam K, Hattrup CL, Kotlarczyk KL, Gutierrez A, Bradley JM, Mukherjee P, Gendler SJ. Tyrosines in the MUC1 cytoplasmic tail modulate transcription via the extracellular signal-regulated kinase 1/2 and nuclear factor-kappaB pathways. *Mol Cancer Res*. 2006;4:489–97.
15. Tsutsumida H, Swanson BJ, Singh PK, Caffrey TC, Kitajima S, Goto M, Yonezawa S, Hollingsworth MA. RNA interference suppression of MUC1 reduces the growth rate and metastatic phenotype of human pancreatic cancer cells. *Clin Cancer Res*. 2006;12:2976–87.
16. Engelmann K, Shen H, Finn OJ. MCF7 side population cells with characteristics of cancer stem/progenitor cells express the tumor antigen MUC1. *Cancer Res*. 2008;68:2419–26.
17. Hikita ST, Kosik KS, Clegg DO, Bamdad C. MUC1* mediates the growth of human pluripotent stem cells. *PLoS One*. 2008;3:e3312.
18. Rowse GJ, Tempero RM, VanLith ML, Hollingsworth MA, Gendler SJ. Tolerance and immunity to MUC1 in a human MUC1 transgenic murine model. *Cancer Res*. 1998;58:315–21.
19. Beatty PL, Narayanan S, Garipey J, Ranganathan S, Finn OJ. Vaccine against MUC1 antigen expressed in inflammatory bowel disease and cancer lessens colonic inflammation and prevents progression to colitis-associated colon cancer. *Cancer Prev Res*. 2010;3:438–46.