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A Knockout for Lynch Syndrome

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Colorectal cancer (CRC) is not a single or uniform disease. Virtually all cancers are caused by genetic “alterations,” but these vary from tumor to tumor. Some investigators have made the unsettling suggestion that each CRC is completely unique, which has major implications for predicting prognosis in individual patients, and complicates the selection of systemic chemotherapies.

It has long been known that only a portion of CRCs respond favorably to adjuvant therapy or to treatments for advanced disease, and it is likely that this is a reflection of genetic heterogeneity among these tumors. At this time, we have only limited insight into how to select patients who will benefit most from these therapies. In an ideal world, we would match the anti-cancer treatment to the tumor, just as we do with antibiotics and infections. However, our current armamentarium of anti-cancer drugs is limited.

Historically, screening for effective drugs begins using in vitro systems. However, the variability among CRC cell lines is such that each may represent a limited proportion of human tumors. Moreover, even if we had a perfect panel of cell lines that represented the complete spectrum of human tumors, in vitro studies might not predict complexities brought about by the host–tumor relationship, which may be more important than the ability of the drug to kill tumor cells per se. Thus, matching treatments to tumors, and developing new cancer drugs, are difficult challenges.

One way to address the host–tumor response is to develop relevant animal models. We have had murine models of familial adenomatous polyposis for some time, in which the animals have germline mutations in the *Apc* gene. The tumors that develop in this model are mainly small intestinal adenomas, but they seem to be similar biologically to sporadic colorectal neoplasms in humans. This model has been useful for developing preventive strategies for recurrent adenomatous polyps, but the insights gained here are not necessarily applicable to all types of human CRC. For example, 15% of human CRCs are deficient in DNA mismatch repair (MMR) activity, have microsatellite instability (MSI), and have unique clinical and pathologic characteristics compared with non-MSI CRCs.¹ Adjuvant chemotherapy for stage III CRC can prevent tumor recurrence in a proportion of patients, but CRCs with MSI respond differently, and show either no benefit² or an increase in mortality when given 5-fluorouracil–based regimens.³ This problem was predicted by the in vitro resistance to a variety of chemotherapeutic agents, including 5-FU, discovered in CRC cell lines with MSI.^{4,5} However, in vitro resistance may not be the entire explanation for the failure of adjuvant therapy in this

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Conflicts of interest

The author discloses no conflicts.

setting. CRCs with MSI are associated with a better natural history, which may be mediated by immune factors, including an increase in tumor-infiltrating lymphocytes. Thus, part of the problem is that the adjuvant treatment may inhibit the immune response more than tumor cell growth.

How do we deal with this challenge? One way would be to develop a murine model of the Lynch syndrome by creating a “knock out” of 1 of the DNA MMR genes. The first attempts at this successfully created mice with defective *Msh2* genes, but the phenotype did not match the Lynch syndrome. Mice with 1 null *Msh2* allele (*Msh2*^{+/-}), which is analogous to having Lynch syndrome, had no excess of early onset tumors. Moreover, the homozygous knockout mice (*Msh2*^{-/-}) were constitutionally MMR deficient and did not develop tumors of the Lynch syndrome spectrum, but were very prone to lymphomas with MSI.^{6,7} This was an interesting model, but was not useful for developing clinical insights into human disease.

To understand the challenge here, one needs to consider the genetics and phenotype of the Lynch syndrome. The disease is caused by inheriting a mutated DNA MMR gene, such as *MSH2*. Affected families follow classic autosomal-dominant Mendelian genetics. However, at a tissue level, the disease acts like a recessive disease; both *MSH2* alleles must be inactivated to lose DNA MMR activity.

As illustrated in Figure 1, HEC59 cells have no wild-type copies of the *MSH2* gene, are DNA MMR deficient, and have MSI. MMR activity can be restored by transferring a single wild-type copy of the *MSH2* gene by the stable transfer of chromosome 2 into the cell line.⁸ Restoration of the MMR system also restores sensitivity to agents that damage DNA.^{4,5} In patients with the Lynch syndrome, the somatic tissues are MMR proficient, but the tumors, which have suffered a “second hit” at the wild-type *MSH2* locus, are MMR deficient. In the rare circumstance where an individual has inherited bi-allelic mutations in *MSH2* (or other MMR gene), there is constitutional MMR deficiency, and an overwhelming diathesis of hematologic cancers and solid tumors in the pediatric years. The homozygous knockout of *Msh2* in the mouse resembles that disease, rather than the classic Lynch syndrome. The reason that the knockout mice do not develop colonic or uterine tumors probably is a reflection of the fact that the proximate genetic targets of MSI that lead to cancer are the somatic mutations in *TGFβRII*, *BAX*, *caspase-5*, and other genes, which have microsatellites in coding exons in humans,⁹ but apparently not in mice.

To help overcome this problem, Kucherlapati et al¹⁰ have created a novel animal model that addresses some, but not all, of these problems. They created a mouse with a conditional knockout of the *Msh2* gene in tissues that express villin, namely, the small and large intestine. This creates a model in which selected cell lineages are completely MMR deficient, while preserving MMR activity in the rest of the organism. These animals do not develop lymphomas; however, they develop adenomas and carcinomas of the intestine. This represents a creative advance, and an opportunity to test preventive and therapeutic strategies for gut malignancies with MSI—something that has not been possible previously. There are limitations to the model. First, villin is expressed throughout the intestine, and the model creates more small intestinal than colonic neoplasms. That may not be a serious problem if one can still use the model to develop clinical insights, such as the response to drug regimens. The other problem is that the mouse tumors may not have mutations at the same target genes as occurs in human tumors, and consequently, the responses to drug interventions may not be fully useful for translational insights. Time will tell.

The authors did 1 more experiment that provided a particularly interesting result. The mouse knockout model permits one to create a “tool box” of unique mice strains, and develop increasingly complex experimental animals. Kucherlapati et al also created a mouse in which

a hypomorphic allele (*Msh2*^{G674D}) was “knocked in,” and the targeted tissues were not necessarily devoid of MMR activity, but might be only partially deficient. Many alterations in human MMR genes in the Lynch syndrome are missense mutations, and when the “second hit” occurs in a somatic tissue, there may be residual but attenuated MMR activity remaining. What would the therapeutic implications of this be? The new model provides an opportunity to find out.

One of the reasons to develop this model was to test the response of tumors to chemotherapeutic drugs, because, as mentioned, the response is different in MSI tumors than in those with proficient MMR activity. The animals that developed intestinal tumors with *Msh2*-null alleles showed no response to systemic treatment with cisplatin or FOLFOX, which is what one might have predicted. Interestingly, the animals with the conditional hypomorphic *Msh2*^{G674D} knock-in had a partial response to the chemotherapy. That was not necessarily what one might have predicted, and represents a novel approach to developing personalized treatments for CRC patients. This observation suggests that we may not only need to know whether our patient’s tumors have MSI, we might need to know the nature of the mutations—both the first and second “hits”—to rationally select the optimal treatment. This development could only have been imagined before the development of this model, and with effort, many additional variations on this theme might be expected in the future. This is surely not the “knock-out” blow to the challenges we face in treating patients with Lynch syndrome tumors, but it opens up an exciting new direction for exploration.

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		Model	DNA MMR status	Phenotype
In vitro		HEC 59 cells (MSH ^{-/-})	MSH2 null, MMR deficient	MSI, tolerant of DNA damage, defective G2/M checkpoint
		HEC 59+chr 2 cells (MSH ^{+/-})	MSH2 restored, MMR proficient	Normal
Mouse		MSH2 ^{+/+}	MMR proficient	Normal
		MSH2 ^{+/-}	MMR proficient, heterozygous mutant at MSH2	Normal
		MSH2 ^{-/-}	Constitutional MMR deficiency	Highly prone to early onset lymphomas; no GI cancers
		MSH2 conditional KO (i.e., VCMsh2 ^{Loxp/null} or VCMsh2 ^{Loxp/G674D})	Tissue specific MMR deficiency	Prone to tumors with MSI in targeted tissues (intestinal)
Human		Lynch Syndrome-MSH2 type	Heterozygous mutant at MSH2	Prone to colon and uterine cancers in mid-life
		Biallelic MSH2 mutations	Constitutional MMR deficiency	Pediatric hematological and solid tumor diathesis

Figure 1.

HEC59 cells were derived from an endometrial cancer cell line, possibly from a patient with Lynch Syndrome-*MSH2* type. Stable transfer of chromosome 2 into the cell line reconstitutes the missing *MSH2* gene, and restores MMR proficiency, sensitivity to cell death after exposure to DNA-damaging agents (including alkylating agents, as well as 5-fluorouracil), and restores the G2/M cell cycle checkpoint, which prevents mitosis when there is unrepaired DNA damage. Importantly, all of this occurs in the presence of just 1 copy of the *MSH2* gene. In the mouse, the presence of a single mutant *Msh2* gene (*Msh2*^{+/-}) is the proper model of the Lynch syndrome, but the mice do not get colonic or uterine tumors in mid-life, as humans do. Mice that are homozygously mutated at *Msh2* (*MSH2*^{-/-}) have constitutional DNA MMR deficiency, with no DNA MMR activity in any tissue, and are highly prone to lymphomas with MSI, but not tumors of the Lynch syndrome spectrum. Patients with Lynch syndrome-*MSH2* type are actually *MSH2*^{+/-} throughout all somatic tissues, but suffer a “second hit” in cells that gives rise to Lynch syndrome tumors, which are *MSH2*^{-/-}. Clinically, the Lynch syndrome is a classic autosomal-dominant genetic disease in which the trait is passed on to half of the offspring. However, the disease is recessive at the tissue level, requiring inactivation of both copies of *MSH2* for DNA MMR deficiency to occur. In this regard, the first mouse models were an imperfect fit for the human disease. However, targeted knockout of the *Msh2* gene in a tissue-specific manner creates tissue specific MMR deficiency and intestinal tumors, which is a closer approximation to the human situation, providing an improved preclinical model to test preventive and treatment strategies.