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Regeneration of articular cartilage in healer and non-healer mice

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Abstract

Mammals rarely regenerate their lost or injured tissues into adulthood. MRL/MpJ mouse strain initially identified to heal full-thickness ear wounds now represents a classical example of mammalian wound regeneration since it can heal a spectrum of injuries such as skin and cardiac wounds, nerve injuries and knee articular cartilage lesions. In addition to MRL/MpJ, a few other mouse strains such as LG/J (a parent of MRL/MpJ) and LGXSM-6 (arising from an intercross between LG/J and SM/J mouse strains) have now been recognized to possess regenerative/healing abilities for articular cartilage and ear wound injuries that are similar, if not superior, to MRL/MpJ mice. While some mechanisms underlying regenerative potential have been begun to emerge, a complete set of biological processes and pathways still needs to be elucidated. Using a panel of healer and non-healer mouse strains, our recent work has provided some insights into the genes that could potentially be associated with healing potential. Future mechanistic studies can help seek the Holy Grail of regenerative medicine. This review highlights the regenerative capacity of selected mouse strains for articular cartilage, in particular, and lessons from other body tissues, in general.

Keywords

MRL/MpJ; LG/J; LGXSM intercross; Tissue repair; Articular cartilage regeneration; Osteoarthritis; Wound healing; Super-healer mice

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Dedication

This work is dedicated to Dr. Dick Heinegård M.D., Ph.D. († May 1, 2013) for his unprecedented work on cartilage and osteoarthritis research.

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1. Introduction

Our research interest focuses on injury and repair of articular cartilage and the relationship between repair (and regeneration) of cartilage and the degeneration seen in osteoarthritis. Recent studies have demonstrated a surprising genetic variability in the capacity for regeneration of cartilage. For these investigations, we and others have turned to lessons provided by studies on regeneration of tissues in mouse models. Tissue regeneration in adult mammals is erratic which has led to renewed interests in classical models of tissue regeneration. Interest in this enduring problem is undergoing a resurgence stimulated by the availability of new mouse strains that are providing insights into the mechanisms that participate in tissue regeneration in mammals. To this end, the MRL/MpJ mouse strain has the exceptional potential to recapitulate regenerative abilities, a characteristic that is otherwise limited to amphibians and organisms other than adult mammals. MRL/MpJ has been demonstrated to regenerate an amazing spectrum of organs and body tissues including the articular cartilage of the knee — a tissue that is notoriously poorly healed. Comparison of MRL/MpJ and other unrelated mouse strains has provided some insights into the types of pathways that lead to regeneration. To provide genetic evidence, we and others have used genetic strains derived from the parent of MRL/MpJ, called LG/J (large), and recombinant inbred strains resulting from mating LG/J with SM/J (small). In this review, we will discuss the healing potential of MRL/MpJ, LG/J and other mouse strains for articular cartilage, in particular, and lessons from other body tissues in general along with some mechanistic insights from the available literature and our recent work.

1.1. Mouse strains

1.1.1. MRL/MpJ—The Murphy Roths Large (MRL) lymphoproliferation (lpr) wild type mouse strain was generated from a series of selective interbreeding of the following strains (the composite genome distribution is indicated in parentheses): C57BL/6J (0.3%), C3H/HeDi (12.1%), AKR/J (12.6%) and LG/J (75%) and then maintained by inbreeding (http://jaxmice.jax.org/strain/000486.html) and (Murphy and Roths, 1979). During the selective breeding of this strain a spontaneous mutation *Fas*^{lpr} was found at generation F_{12}, which was associated with a major defect in immune regulation (Adachi et al., 1993). The MRL/lpr mouse strain was originally selected for its large body size (body weight) and was mainly used as an autoimmune model. In contrast to MRL/lpr, the MRL/MpJ (MRL derived by the Murphy group of Jackson Laboratory) mice carry a normal (wild type) Fas gene. In spite of carrying the normal Fas gene, MRL/MpJ still exhibits autoimmune disorders; however, the symptoms are manifested later in life compared to MRL/MpJ-Fas^{lpr} mice. MRL/MpJ-Fas^{lpr} and the MRL/MpJ mice are kept congenic with each other by back-crosses to the MRL/MpJ wild type every 5–10 inbred generations.

1.1.2. Recombinant inbred lines from LG/J and SM/J—The LG/J mouse strain, a parent of MRL/MpJ, was selected for its large body size while another strain namely SM/J was selected for its small body size in two separate experiments performed in the first half of the 20th century. Both strains have been maintained by brother–sister mating for over 200 generations. These two inbred strains have obvious differences in body size and growth and are also known to differ in a wide range of other traits (Hrbek et al., 2006). The LGXSM

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intercross is a model for studying the genetics of complex traits segregating many interacting genes of small effect. Each LGXSM recombinant inbred strain is a unique recombination of the parental genomes. As in ordinary inbred strains, recombinant inbred strains are genetically identical, but each strain is a different 50:50 mix of the parental genotypes so that phenotypic and genotypic differences between strains allow gene mapping to a 10 cM (centimorgan) resolution containing a few hundred genes (Hrbek et al., 2006). We now have 45 recombinant inbred strains (JM Cheverud, personal communication). The ear wound healing and articular cartilage regeneration phenotypes studied in recombinant inbred strains and in several common inbred mouse strains will be highlighted in this article.

2. MRL/MpJ healing

Since 1998, first serendipitously discovered for their rapid ability to heal 2-millimeter through-and-through external ear wounds, MRL/MpJ mice have gained substantial popularity among investigators interested in tissue regeneration in mammals (Clark et al., 1998). Then, over the course of the next decade several investigators reported the reproducibility of ear wound healing in this mouse strain (McBrearty et al., 1998; Kench et al., 1999; Masinde et al., 2001; Gourevitch et al., 2003; Rajnoch et al., 2003; Davis et al., 2005; Beare et al., 2006; Colwell et al., 2006; Metcalfe et al., 2006; Naseem et al., 2007; Fitzgerald et al., 2008; Rai et al., 2012). After the discovery of this exceptional healer mouse strain, it was natural for other investigators to explore whether tissues other than external ears also regenerate in MRL/MpJ mice. Accelerated regeneration in amputated digit tips (Han et al., 2005; Chadwick et al., 2007), peripheral nerves (Buckley et al., 2011), alkali-burned corneas (Ueno et al., 2005), and cardiac wounds (Leferovich et al., 2001; Bedelbaeva et al., 2004; Naseem et al., 2007) has been shown in MRL/MpJ mice. In contrast to the ear wound phenotype, surgically induced skin wounds on the dorsum did not show accelerated healing. All four studies conducted so far (Beare et al., 2006; Colwell et al., 2006; Metcalfe et al., 2006; Buckley et al., 2011) have collectively shown that there was slow re-epithelialization without the formation of hair follicles and sebaceous glands, along with the formation of granulation tissue and scarring at the site of injury. A summary of regenerative phenotypes in MRL/MpJ mice is provided in Table 1.

Two other phenotypes (articular cartilage regeneration and intraarticular fracture healing) are discussed separately.

3. Other mouse strains that exhibit super-healing potential

Although being called a super healer mouse (Heydemann, 2012), other strains can also heal cartilage damage. LG/J, which shares 75% of its genome with MRL/MpJ (Murphy and Roths, 1979) and LGXSM-6 which shares 76% of its genome with LG/J (Hrbek et al., 2006) have been shown to possess greater regenerative potential for ear wound healing than SM/J, C57BL/6J and many of the other LGXSM recombinant inbred mouse strains (Rai et al., 2012). In addition, the African spiny mouse (Acomys) originally found to be capable of shedding and regenerating portions of its skin exhibited complete regeneration of ear wounds including hair follicles, sebaceous glands, dermis and cartilage (Seifert et al., 2012). The process also involved accelerated reformation of the outer layer of the skin by migrating

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epidermal cells. A common attribute of species with regenerative ability is that they have mechanisms to promote regeneration in lieu of fibrosis and scarring, thus allowing the regenerative tissue (a blastema) to continue on the pathway of differentiation.

4. Genetic variability in articular cartilage regeneration

Articular cartilage is notoriously poor at healing: a partial thickness wound never heals and a full-thickness wound rarely heals. Articular cartilage is a specialized tissue that resides on the articulating surface of long bones forming the bearing surfaces of these joints. The superficial zone hyaline articular cartilage provides a smooth, wear-resistant articulating surface. At the same time, articular cartilage per se is a simple tissue, which is deficient in blood and lymphatic vasculature and nerve supply. It is composed of only one cell type, namely chondrocytes, embedded in a relatively homogeneous extracellular matrix (Lyons et al., 2006; Hollander et al., 2010). The extracellular matrix of articular cartilage consists of an intricate network containing predominantly fibrillar proteoglycans and collagens that confer properties on cartilage that are distinct from other connective tissues. The proteoglycans are large hydrophilic aggregates and, in articular cartilage, the dominant structural proteoglycan is aggrecan. Among several collagen types, collagen type II, collagen type IX and collagen type XI comprise a fibrous framework (Poole et al., 2001), collagen type VI is present pericellularly (i.e. around the cartilage cells) and collagen type X is present in the calcifying zone of the articular cartilage (Poole et al., 1991). The structural qualities of articular cartilage and the high ratio of matrix to cells allow it to function as a lubricated shock absorber, but provide a challenge to healing or regeneration of the tissue.

While articular cartilage repair phenotypes in humans are not accessible, work on mouse models has begun to generate some important data. Thus far, two laboratories have looked at healing of full-thickness articular cartilage lesions in MRL/MpJ mouse strain. A set of experiments pioneered by Fitzgerald and colleagues (Fitzgerald et al., 2008), demonstrated that MRL/MpJ mice could regenerate articular cartilage defects significantly better than those in C57BL/6 mice. These investigators tested full- as well as partial-thickness lesions on the trochlear groove articular cartilage of mice using both sexes. The healing outcomes were gauged at two time points (6- and 12-weeks) following surgery through an established scoring criterion (Wakitani et al., 1994). It was found that at both time points there was a superior healing response in MRL/MpJ mice (but limited to full-thickness lesions only) with abundant chondrocytes and an extracellular matrix rich in proteoglycan, collagen II and collagen VI at the injury site. In contrast, the C57BL/6J mice failed to regenerate articular cartilage tissues. Interestingly, these investigators also studied healing of 2-millimeter ear wounds in the same mice in which articular cartilage defects were created in an effort to analyze possible correlations between the regenerative abilities of ear wounds and articular cartilage lesions. It was observed that there is a slight positive correlation between ear wound healing and articular cartilage regeneration (Fitzgerald et al., 2008).

We have recently shown a variation in the magnitude and extent of articular cartilage regeneration potential in MRL/MpJ and several related and unrelated mouse strains from common inbred and recombinant inbred lines (Rai et al., 2012). Like MRL/MpJ, the LG/J mouse strain also displays complete healing of the ear wounds, including the ear cartilage

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(Blankenhorn et al., 2009). This raises the possibility that, similar to MRL/MpJ mice, the articular cartilage may also heal in LG/J mice. To investigate this possibility, we examined the extent of cartilage regeneration in a set of common inbred mouse strains, including both healers and non-healers, and in a set of LGXSM recombinant inbred lines formed from the intercross of the LG/J (healer) and SM/J (non-healer) inbred mouse strains. The conceptual starting point for recombinant inbred strains is that any differential phenotype can be attributed to the genetic background of the mice (as confirmed by broad-sense heritability estimates) and a restricted set of genes underlying regeneration can be identified according to the way they have been inherited from parental strains. Therefore, we took the approach of Fitzgerald and co-workers (Fitzgerald et al., 2008) to create full-thickness cartilage wounds on the trochlear groove of distal femur in genetic mouse models. There were significant differences among the inbred mouse strains in the type of cartilage formed at the site of injury (varying from typical hyaline articular cartilage to fibrous or no cartilage tissue at all), staining intensity of proteoglycan contents (intense staining to no staining), surface regularity of the injured area (smooth surface to extremely irregular), integration status of the injured area with the native health cartilage (both edges integrated to at all no integration) and finally thickness of the repair zone (in par with the native cartilage to no integration). Based on these criteria, we have listed mouse strains that have super (LG/J, LGXSM-6, MRL/MpJ), intermediate (LGXSM-5, LGXSM-35) and poor (C57BL/6J, SM/J, DBA/1J, DBA/2J, LGXSM-33) healing capability for articular cartilage (Fig. 1). Thus, we were able to identify and report, for the first time, several new mouse strains with an impressive spectrum of regenerative ability (from superb healers to intermediate healers to non-healers): in addition, our study confirmed and extended the findings of Fitzgerald and co-workers (Fitzgerald et al., 2008) in MRL/MpJ mouse strain. In addition to phenotypic characterization, our study has also provided important insights into the genetics of cartilage regeneration after injury.

While a previous study showed weak, but positive, correlation between ear wound healing and articular cartilage regeneration (Fitzgerald et al., 2008), we (Rai et al., 2012) found a strong positive correlation between ear wound healing in common inbred as well as recombinant inbred mouse strains indicating some similar underlying mechanisms of healing.

In summary, the outcomes from the above two studies suggest that MRL/MpJ, LG/J and LGXSM-6 possess an exceptional healing ability to repair full-thickness articular cartilage injuries (in fact LG/J and LGXSM-6 heal their articular cartilage better than MRL/MpJ) while other strains did not heal as well.

5. Intraarticular fracture healing in MRL/MpJ mice

While work on articular cartilage regeneration in MRL/MpJ mice is scarce, here we reviewed another important, yet related, phenotype of intraarticular fracture healing. Experiments in MRL/MpJ mice demonstrated that these mice exhibit improved bone and cartilage healing after non-invasive intraarticular fracture (Ward et al., 2008). The most significant findings from this study were the following: (i) the control C57BL/6 mice showed lower bone density and higher subchondral bone thickness, (ii) the C57BL/6 wild

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type (non-healer) mice showed augmented cartilage degeneration after intraarticular fracture compared to MRL/MpJ mice and (iii) the fractured limb of MRL/MpJ was essentially similar in characteristics compared to a contralateral non-fractured limb indicating a structural and functional restoration of fractured tissues. Taken together, these findings suggest that MRL/MpJ mice, which possess superior healing for bone and articular cartilage fractures, are protected from developing post-traumatic osteoarthritis since it is believed that post-traumatic osteoarthritis is a frequent long-term complication of intraarticular fractures (Buckwalter and Brown, 2004).

6. Cartilage repair, osteoarthritis and autoimmunity

In the laboratory of Francesco Dell'Accio, it was found that the C57BL/6 strain does not heal a full-thickness articular cartilage defect, but the DBA/1 strain does (Eltawil et al., 2009). These investigators did not test the MRL/MpJ mouse per se, but have shown DBA/1 as a relatively fast healing. Longitudinal full-thickness articular cartilage injuries were generated in the patellar groove of these two mouse strains by the use of a custom made device in which a glass bead was placed approximately 200 μm to the tip ofa 26 gauge needle. The tip of the needle was placed anteriorly to the intercondylar notch and gently moved along the entire length of the patellar groove. The most significant findings were (i) 8-week old DBA/1 mice displayed consistent superior healing of the articular cartilage defect than C57BL/6, (ii) DBA/1 mice showed a significantly less cell death and cell proliferation than C57BL/6 and (iii) most importantly, the increase in articular cartilage repair was correlated with the development of osteoarthritis; older (8-month) DBA/1 mice failed to repair, but unlike age-matched C57BL/6, they had no signs of osteoarthritis. Once the cartilage of the superficial zone starts to deteriorate, though, osteoarthritis sets in, triggering an irreversible process that eventually leads to the loss of underlying layers of cartilage.

We failed to observe a regenerative response in DBA/1J and DBA/2J mouse strains for articular cartilage lesions (Rai et al., 2012) possibly owing to differences in the experimental approach as well as genetic drift that may have occurred over the course of hundreds of generations of separation. However, we have observed regenerative responses and varying susceptibility to post-traumatic osteoarthritis in other strains. We used two strains from LGXSM intercross namely LGXSM-6 (with a super-healing ability for articular cartilage and ear wounds) and LGXSM-33 (a poor healer for articular cartilage and ear wounds) to induce post-traumatic osteoarthritis through destabilization of the medial meniscus (Hashimoto et al., 2012). Analysis of articular cartilage and bone changes following surgical induction of osteoarthritis showed that the healer strain (LGXSM-6) was significantly protected from developing osteoarthritis-like change in bone and cartilage while non-healer strain (LGXSM-33) was susceptible to post-traumatic osteoarthritis. Thus, our findings of a negative correlation between ability for articular cartilage regeneration and susceptibility to post-traumatic osteoarthritis augment the findings from DBA/1 and MRL mouse strains (Ward et al., 2008; Eltawil et al., 2009).

Both MRL/MpJ and LG/J are susceptible to autoimmune disorders (Peng et al., 1996) as well as have the potential to heal body tissues. This might indicate a common genetic

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predisposition to these phenotypes although experimental work failed to provide an evidence for a genetic link between the two phenotypes (Kench et al., 1999). In addition, MRL/MpJ mice exhibit diminished levels of inducible proinflammatory cytokines in response to lipopolysaccharide stimulation (Kench et al., 1999). To examine the relationship between inflammation and post-traumatic osteoarthritis, a recent study found that MRL/MpJ mice with a lower level of inflammation are protected from post-traumatic osteoarthritis suggesting a link between joint tissue inflammation and osteoarthritis progression (Lewis et al., 2013).

7. Unique properties of super-healer mice

In those injury cases where the MRL/MpJ or LG/J background have been shown to exhibit profound healing potential, numerous mechanisms have been identified (Table 2) that begin to explain this phenomenon including decreased scar formation, amplified inflammatory response (Ueno et al., 2005; Thuret et al., 2012), reduced apoptosis (Naseem et al., 2007), increased proliferation (Naseem et al., 2007), cell differentiation (Thuret et al., 2012), improved remodeling (Clark et al., 1998), enhanced stem cell function (Leferovich et al., 2001; Naseem et al., 2007) and general up-regulation of genes involved in repair including angiogenesis, DNA repair and replication, protein biosynthesis, glycolysis and cell adhesion (Thuret et al., 2012). Data generated from genetic analysis has further revealed that several other mechanisms are also associated with enhanced regeneration since over 40 different genetic loci associated with ear wound healing phenotypes have been identified (Heber-Katz et al., 2004; Heydemann, 2012). A role for circulating cytokines and cells in MRL/MpJ ear wound regenerative response has also been suggested based on the evidence of accelerated healing response following a secondary wound near a primary wound (Davis et al., 2005). While these studies suggest that several biological processes alone or in concert with other processes are instrumental to healing ability, they lack the information on which particular genes or pathways are involved that make the MRL/MpJ mouse so unique.

8. Emerging molecular mechanisms of regeneration

As mentioned, many of the mice able to regenerate structures have decreased fibrosis and scarring, mechanisms that promote healing of the wound through generation of a basement membrane, which is thought to inhibit regeneration of the tissues. The molecular mechanisms orchestrating a regenerative potential in a wide range of tissues in certain mouse strains have begun to emerge in recent years. It has been found that elevated expression of matrix metalloproteinases is linked to regenerative potential in the retina of the MRL/MpJ mouse (Tucker et al., 2008). These authors suggested that the higher levels of *Mmp2, Mmp9* and *Mmp14* expression and subsequent decreased inhibitory extracellular matrix molecules (e.g. neurocan and *Cd44*) provide an environment conducive to regeneration in the retina and help to degrade and remove inhibitory basement membrane.

In another study, genome-wide chip microarray analysis of RNA isolated from digit amputation site has shown that several of the keratin genes (including *Krt6* and *Krt16*) were differentially expressed between MRL/MpJ and C57BL/6 mice (Cheng et al., 2013). Immunohistochemical analysis of *Krt6* and *Krt16* in amputated digits and ear wounds

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further confirmed that higher expression of these genes is associated with the regeneration potential suggesting that normal MRL/MpJ skin is in a keratinocyte-activated state, which may provide it with a superior response to regeneration. Similarly another group of investigators undertook a microarray study on healing of digit amputation in MRL/MpJ mouse and found that genes and pathways related to *Bmp* and *Tgfb/Igf* signaling were modulated in the healer strain (Chadwick et al., 2007).

Studying ear wounds, it has been shown that MRL/MpJ mice carry some characteristics that are most common to mammalian embryonic stem cells and classical regenerators (Bedelbaeva et al., 2010) thus providing some clues for their regenerative potential. Among these characteristics the most significant are: i) fibroblast-like cells exhibit a distinct cell-cycle (G2/M accumulation) phenotype, ii) persistent levels of intrinsic DNA damage, iii) a heightened basal and wound site DNA damage/repair response and iv) lack of *p21* expression. The pronounced intrinsic DNA damage in cells derived from healer strains was strikingly consistent with other reports showing that mouse embryonic stem cells display endogenous DNA damage and a faulty G1 checkpoint (Hong and Stambrook, 2004; Galvin et al., 2008). In those reports, a lack of check-point control was attributed in part to a lack of *p21* induction. Therefore, the laboratory of Ellen Heber-Katz could turn a non-healer mouse strain to a healer for ear wounds by deleting the *p21* gene (Bedelbaeva et al., 2010). *Mmps* activity and matrix remodeling after injury have also been found to be associated with ear wound healing response in MRL/MpJ mice (Gourevitch et al., 2003).

While the above studies have focused on unraveling the mechanisms of tissue regeneration in tissues other than the articular cartilage, a recent study has revealed some of the important genes significantly correlated with the healing potential (Rai et al., 2013). This study took advantage of archived histological sections from recombinant inbred mouse strains for the analysis of a large set of candidate genes through branched-chain DNA technology directly from tissue lysates (prepared from the multiple joint tissues (including cartilage, subchondral bone, meniscus, synovium, joint capsule, and growth plate). Pearson correlation of candidate genes showed that several genes were correlated with both ear wound and knee articular cartilage regeneration phenotypes in a range of mouse strains with different capacities for healing. Interestingly, genes representing DNA repair (*Xrcc2, Axin2*) and Wnt signaling pathway (*Axin2, Wnt16*) were significantly positively correlated with both phenotypes, which hint towards a common underlying mechanism for regeneration of two tissue types in mice. Additional bio-informatic assessment of allelic polymorphism within or near the candidate genes and heritability estimates further strengthen the findings on the differential gene expression between healer and non-healer mouse strains. A summary of these emerging mechanisms is provided in Table 3.

Taken together, the data generated so far are insufficient to demonstrate conclusively the molecular biology of the healer mice for articular cartilage regeneration. However, recent yet exciting data coming from genetic studies of tissue regeneration along with some molecular insights into the molecules that underlie the exceptional regenerative capability in mice have begun to unfold the mystery of why a super healer mouse could carry the regenerative ability for articular cartilage (and other tissues) into adulthood. The availability of genetic mouse strains with varying potential for tissue repair mirrors the variation to

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repair following injury in diverse human populations. Therefore, in the near future, these mouse resources would be valuable tools to find candidate genes that contribute to the variability of cartilage regeneration and osteoarthritis in human.

9. Conclusions

1. MRL/MpJ is indeed a super healer mouse, which can heal a broad range of injuries (ear wounds, skin wounds, cardiac wounds, nerve injuries, articular cartilage lesions and intraarticular fractures).
2. In addition to MRL/MpJ, there are few other mouse strains that possess similar, if not superior, articular cartilage and ear wound healing capability and include LG/J and LGXSM-6.
3. While some mechanisms have been proposed that appear to participate in the super-healing ability, a complete set of biological processes needs to be determined.
4. For articular cartilage a limited set of genes (*Wnt16*, *Axin2*, *Xrcc2* and *Pcna*) have been associated with the superior healing in a number of healer mouse strains.

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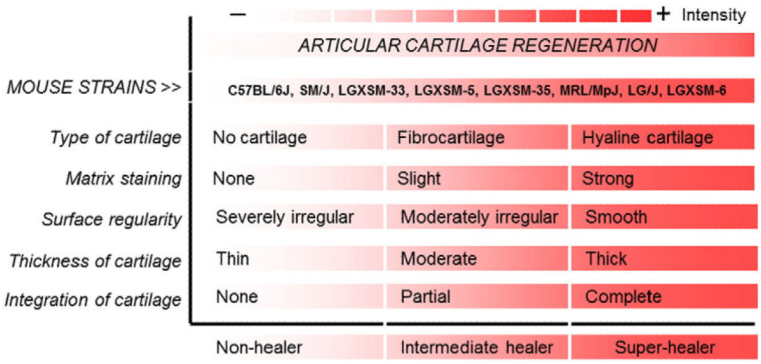

Fig. 1.

Summary of strains with and without healing potential for articular cartilage lesions. Based on our recent work on articular cartilage regeneration we have listed the mouse strains in order of their healing potential for full-thickness articular cartilage lesions (Rai et al., 2012) using a cartilage repair scoring system modified from Shigeyuki Wakitani (Wakitani et al., 1994).

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Table 1

Summary of regenerative phenotypes reported in MRL/MpJ mice.

Phenotype	Outcome	Reference
Ear wound healing	Complete closure along with full restoration of all structures of 2-millimeter diameter wounds in the ear pinna	(Clark et al., 1998; McBrearty et al., 1998; Kench et al., 1999; Masinde et al., 2001; Gourevitch et al., 2003; Rajnoch et al., 2003; Davis et al., 2005; Beare et al., 2006; Colwell et al., 2006; Metcalfe et al., 2006; Naseem et al., 2007; Fitzgerald et al., 2008; Rai et al., 2012)
Digit tip regrowth	Digit tip amputated neonatally happened to regrow along with complete structural and functional restoration (including nail)	(Han et al., 2005; Chadwick et al., 2007)
Peripheral nerve regeneration	Higher proximal wound nerve density	(Buckley et al., 2011)
Alkali-burned cornea	Rapid re-epithelialization along with restoration of complete functional capacity of the eye without any loss of corneal transparency	(Ueno et al., 2005),
Cardiac wound	Accelerated healing, increased mitosis, increased function by echo, less collagen deposition along with restoration of the function	(Leferovich et al., 2001; Bedelbaeva et al., 2004; Naseem et al., 2007)
Articular cartilage regeneration	Significant regeneration of full-thickness articular cartilage lesions with maximum restoration of matrix staining and hyaline nature of cartilage	(Fitzgerald et al., 2008; Rai et al., 2012)
Intraarticular fracture	Rapid fracture healing along with decreased cartilage degeneration	(Ward et al., 2008)
Surgical skin wound	Slow re-epithelialization, absence of hair follicles and sebaceous glands	(Beare et al., 2006; Colwell et al., 2006; Metcalfe et al., 2006; Buckley et al., 2011)

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Table 2

Summary of potential biological processes associated with tissue regeneration in super healer mice.

Injury setting	Possible biological process	Reference
Alkali-burned cornea	Reduced inflammation and fibrosis, rapid re-epithelialization	(Ueno et al., 2005)
Cardiac cryoinjury	Increased cellular proliferation and vasculogenesis, decreased apoptosis	(Naseem et al., 2007)
Ear wound healing	Increased cell proliferation and migration, increased angiogenesis and extracellular matrix production	(Clark et al., 1998)
Ear wound regeneration	Increased tenascin expression	(Naseem et al., 2007)
Heart regeneration	Increased cell proliferation	(Leferovich et al., 2001)
Ear wound healing	Cytokines	(Davis et al., 2005)
Spinal cord injury	Angiogenesis, DNA replication, protein biosynthesis, glycolysis and cell adhesion	(Thuret et al., 2012).

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Table 3

Summary of emerging mechanisms for tissue regeneration in super healer mice.

Regeneration phenotype	Emerging mechanism	Reference
Retina injury	Elevated expression of Mmp2, *Mmp9* and *Mmp14*, repressed expression of neurocan and *Cd44*	(Tucker et al., 2008)
Digit amputation	Higher expression *of Krt6* and *Krt16*, up-regulation of Bmp and Tgfb/Igf pathways	(Cheng et al., 2013) (Chadwick et al., 2007)
Ear wound	Lack of p21 expression, increased DNA damage, increased *Mmp2* expression, lowered *Timp3* expression	(Bedelbaeva et al., 2010) (Gourevitch et al., 2003)
Ear wound/articular cartilage lesions	Higher expression of DNA repair *(Xrcc2, Pcna)* and Wnt signaling (*Axin2, Wnt 16)* genes	(Rai et al., 2013)