Rodent-specific rearrangement between hyaluronidase and chemokine receptor gene clusters and its implication in cancer resistance, inflammation and aging

A dissertation submitted for partial fulfillment of BS-MS dual degree in Science

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Certificate of Examination

This is to certify that the dissertation titled "Rodent-specific rearrangement between hyaluronidase and chemokine receptor gene clusters and its implication in cancer resistance, inflammation and aging" submitted by Anjoom Thahir.A.V (Reg No: MS13139) for the partial fulfillment of BS-MS dual degree program of IISER Mohali has been examined by the thesis committee duly appointed by the institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

Dr. Rajesh Ramachandran	Dr. Shashi Bhushan Pandit	Dr. Kuljeet Singh Sandhu
(Committee Member)	(Committee Member)	(Supervisor)

Dated:

Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr. Kuljeet Singh Sandhu at the Indian Institute of Science Education and Research Mohali. This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgment of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

Anjoom Thahir.A.V
(Candidate)
Dated: April 20th, 2018

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Kuljeet Singh Sandhu
(Supervisor)

Acknowledgment

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Anjoom

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Abstract

Cancer is a condition when abnormal cells divides in an uncontrollable manner. Both environment and genetic factors have equal role to play in developing cancer. Cancer is widespread across animal kingdom. But few large animals (elephants, whales etc.) and smaller rodents (naked mole rat, blind mole rat etc.) are extremely resistant to cancer. Elephant genome has lot of copies of tumor suppressor gene, p53, mediate cancer resistance. But in case of naked mole rat, cancer resistance is attributed to the high amounts of High molecular mass hyaluronan in the extra cellular matrix. Recent study reported that oncogene with a neighboring tumor suppressor gene is less prone to amplification. So, it will be interesting to know whether genomic rearrangement near oncogenes or tumor suppressor genes have any role in naked mole rat's cancer resistance.

In this study, we have observed a single large rearrangement between hyaluronidase and chemokine receptor gene clusters in rodents. Interestingly it was observed that the organisms, naked mole rat and guinea pig, showing cancer resistant properties have significant long-range rearrangement. The rearrangement is happening exclusively in rodents. It is well reported that chemokine receptors and hyaluronidase are involved in inflammation, cancer progression and aging. Owing to the above knowledge, we suspect that the rearrangement might explain rodents' survival in stressful underground habitat and their ability to develop or resist cancer and their varying lifespan. It will be interesting to know how the chemokine signaling pathway differs regarding chemokine receptor gene expression in different rodents in relation to their physiological and environmental factors. We further hypothesize that 3D chromatin interactions and epigenetic modifications near chemokine receptor gene cluster may vary in time (different stages of aging and cancer) and space (different cells involved in tumor micro environment and inflammatory signaling pathway). Studying involvement of tumor micro environment, in terms of chemokine-chemokine receptor mediated interactions will help in understanding cancer in a broader sense.

Keywords: Chemokine signaling, Hyaluronidase, Immunity, Inflammation, Cancer, Aging, Naked Mole Rat, Chromosome rearrangement, Rodents, Tumor micro environment, Cancer-associated fibroblast, Epigenetic modifications.

Introduction

Tumor microenvironment

The tumor microenvironment (TME) has a major role in cancer development and progression. TME (Figure:1) consist of stromal cells (angiogenic vascular cells, cancer-associated fibroblasts (CAF) and infiltrating immune cells) together with chemokines, growth factors and many other factors released by stromal cells and other cells in the surroundings and extra cellular matrix (ECM) (1). The interactions of cancer cells with TME are essential for tumorigenesis, proliferation and metastasis (2).

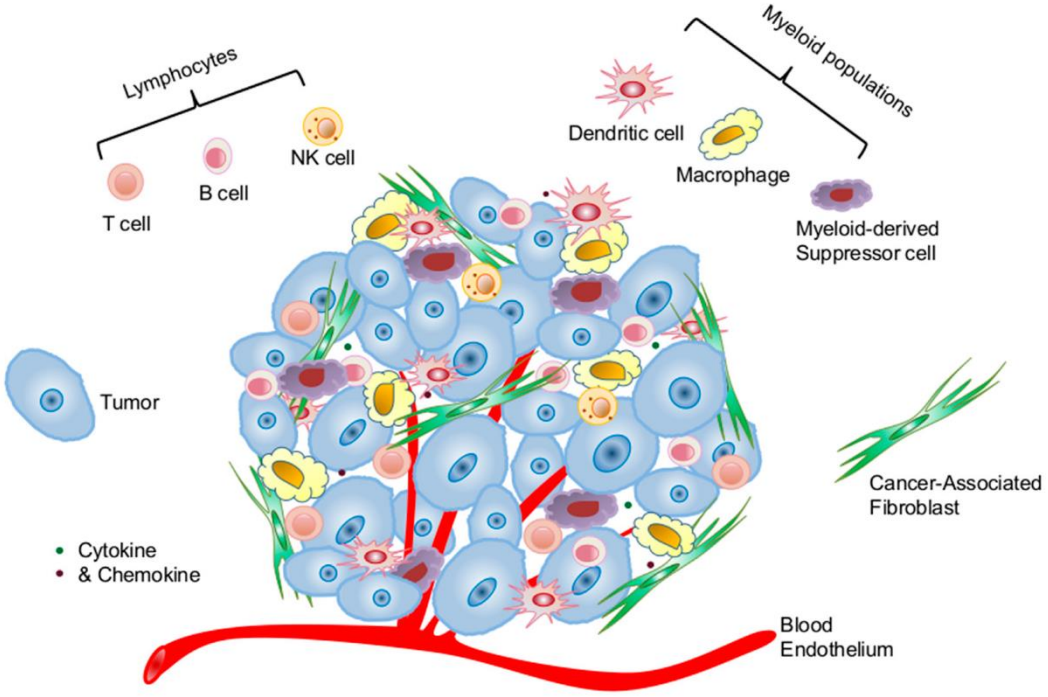

Figure: 1

There are many different cells involved in tumor microenvironment. The constant cross talk between fibroblast and different immune cells this region via many signaling molecules (including chemokines) will mediate cancer initiation, progression and metastasis. (72)

Hyaluronan

Hyaluronan (HA) is one of the major ECM polysaccharides found mostly in soft connective tissues. Under homeostatic conditions, in humans and mouse, HA exists as a high molecular weight polymer (HMW-HA >10^6 Da) non-sulfated glycosaminoglycan consisting of repeating subunits of (β,1-4)-D-glucuronic acid-(β,1-3)-N-acetyl-D-glucosamine, that has important roles in tissue structural integrity of extracellular matrix (3). HA is synthesized by hyaluronan synthases (HAS1,2,3) and degradation is mediated by six hyaluronan degrading enzymes – hyaluronidase 1-6 (HYAL1-6) (4).

Depending on the polymer size, HA appears to have distinct biological functions in different cells that it interacts with (5). During a tissue injury or related stress, HMW-HA is fragmented into low molecular weight HA (LMW-HA). Generally, HMW-HA does not induce inflammatory or proliferative genes (6). Studies using different inflammatory leukocytes have shown that fragmented LMW-HA in the range of 2.5×10^5Da, can induce the expression of inflammatory genes (7,8,9). Malignancy is reported in many cases when HYAL is overexpressed (10,11,12,13,14,15) And if HA is accumulated, it decreases the tumorigenic potential (16,17,18,19,20). These studies support that LMW-HA induces inflammatory response and tumorigenesis. However, many studies show that overproduction of HA is associated with increased malignancy and poor survival. (21,22,23,24,25). So, there is no discrete margin to claim that levels of HA in tumor malignancy and survival.

Apart from HYAL genes, both reactive oxygen species (ROS) and UV light degrade high to low molecular weight HA (26,27). LMW-HA triggers chemokine synthesis and activation of macrophage and thereby a pro-inflammatory response (28,29). It is also reported that degradation of HA during tissue injury will trigger an immune response (30). So, HA is degraded either by injury or ROS (in case of stress) and it will result in inflammation.

CD44 is an HA-binding protein, and HA-CD44 interactions play an important role in development, inflammation, T cell recruitment and activation, and in tumor growth and metastasis (31,32). Expression of CD44 is found mostly in stromal cells such as fibroblasts and smooth muscle cells, epithelial cells and immune cells such as neutrophils, macrophages and lymphocytes (33).

Chemokine signaling

Chemokines are a subset of signaling molecule, cytokines secreted by all immune cells. These immune cells use chemokine gradient (chemotaxis) as the mechanism for movement and localization. There are about 50 types of chemokines identified so far. According to NH2-terminal cysteine-motifs, these chemokines are classified into four subfamilies: CXC, CC, CX3C and XC (34,35,36). All of these chemokines are involved in cell signaling by chemokine receptors (CCRs), which are seven domain transmembrane G protein coupled receptors. Unlike chemokines, only about 20 CCRs mediate chemokine signaling in cells and are divided into four classes, named according to the type of chemokine (CC, CXC, CX_3C or XC) that they bind with.

A new system of classification based on physiological features, including conditions and locations of chemokine production and cellular distribution of chemokine receptors, divides chemokines mainly into two categories. Inflammatory or inducible chemokines and homeostatic (constitutive, housekeeping or lymphoid) chemokines. Upon stimulation by pro-inflammatory cytokines or during contact with pathogenic agents, resident and infiltrated cells express inflammatory chemokines. These include CXCL-8, CCL2, CCL3, CCL4, CCL5, CCL11 and CXCL10 (37). They are specialized in recruitment of effector cells, including monocytes, granulocytes and effector T-cells. Homeostatic chemokines, in contrast, is involved in leucocyte navigation during hematologists in the bone marrow and thymus, during initiation of adaptive immune responses in the spleen and lymph nodes, and in immune surveillance of healthy peripheral tissues. These include CCL14, CCL19, CCL20, CCL21, CCL25, CCL27, CXCL12 and CXCL13 (38). Some have dual functions too. (39,40).

Like chemokines, CCRs can also be grouped as inflammatory receptors and homeostasis (41). CCRs can be categorized loosely into two based on their breadth of expression. Such as those expressed exclusively on a small number of leukocyte and those that are more broadly expressed (42). Depending upon the chemokines and chemokine receptors involved, the pattern of lymphocyte migration throughout their life cycle (lymphopoiesis, antigen-dependent priming, inflammation and immune surveillance) is fine-tuned (39).

Figure:2 shows the chemokine-chemokine receptor families. Figure:3 shows chemokine-CCR interaction in cancer metastasis and progression.

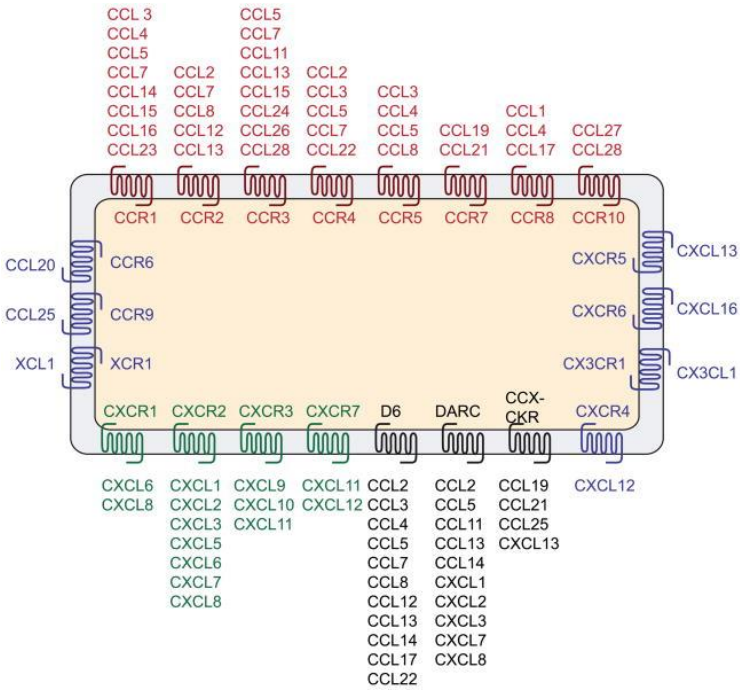

Figure: 2

Chemokines and chemokine receptor family (73). Four families of chemokines and chemokine receptors are characterized.

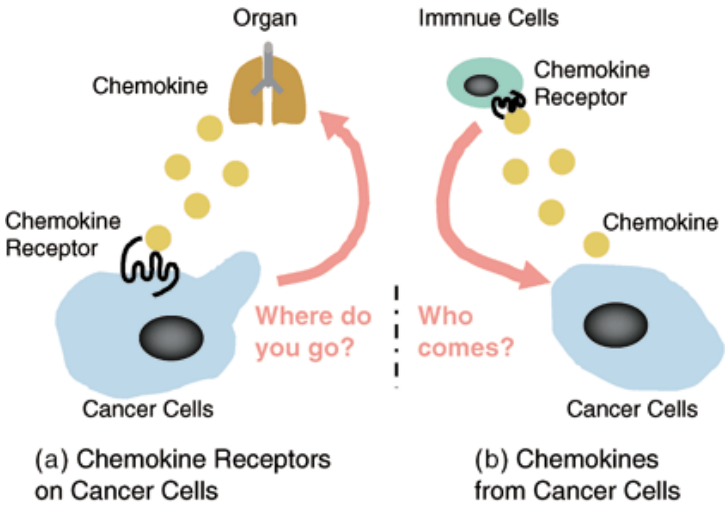

Figure: 3

(a) Chemokine receptors expressed on cancer cells in cancer metastasis.

(b) Chemokines derived from cancer cells in cancer progression (46).

Cancer tissue consists of both cancer cells and various stromal cells, and leukocytes that infiltrate into cancer are of particular importance in cancer progression. So chemokine signaling will be crucial for the cross-talk in cancer environment. There are overwhelming evidence showing the increased expression of chemokines and CCRs in cancer tissues is correlated with malignancy and less survival (43,44,45,46,47,48,49). And studies in human T cells show association with CCR expression and aging (50). All these results suggesting a novel role of chemokine signaling in inflammation, cancer and aging. Figure: 4 shows possible hyaluronan degradation pathway.

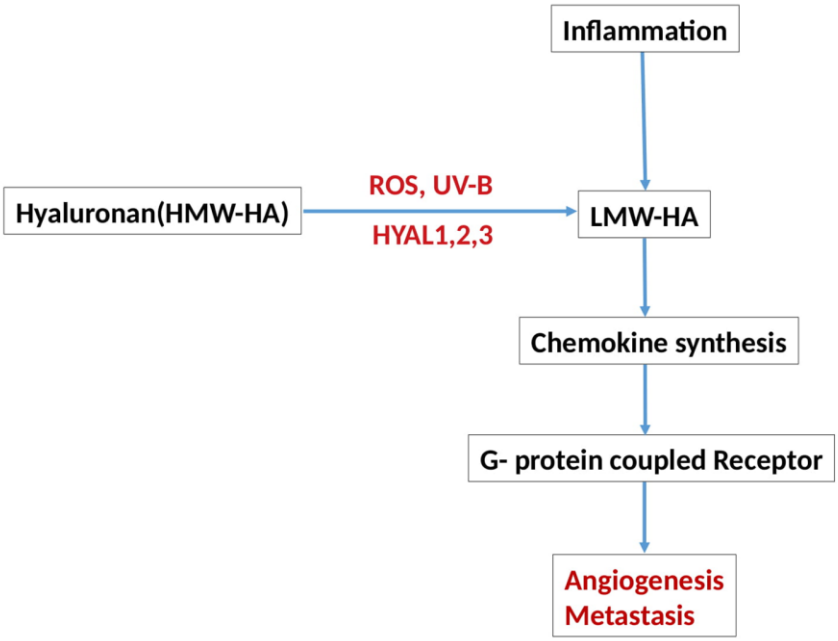

Figure: 4

Possible mechanisms of HA degradation. Inflammation and HYAL can degrade the HMW-HA to LMW-HA which triggers a GPCR mediated signaling.

Naked Mole Rat and cancer

Naked mole rat (NMR), *Heterocephalus glaber,* is a sub-Saharan burrowing rodent with wrinkled skin and deformed skull. They live in large colonies and are less sensitive to pain (51). They are virtually blind and have no hair. This poop eating eusocial animal is shown to survive up to 18 minutes of anoxia and can tolerate hours of extreme hypoxia (52). They have low body temperature (30.0-32.0 degrees C) and poor thermoregulatory ability when compared to other mammals (53). Apart from their extreme lifespan (up to 30 years) compared to other similar sized mammals, they are well known for their cancer resistance (54,55,56,57,58).

HMW-HA secreted by NMR fibroblasts is extremely heavy (over five times larger). This very HMW-HA mediates cancer resistance in NMR (59). HMW-HA represses mitogenic signaling and has anti-inflammatory properties (60). Due to the decreased activity of HA-degrading hyaluronidase enzymes (HYAL1,2,3) and a unique sequence of hyaluronan synthase 2 (*HAS2*), NMR tissues will accumulate very HMW-HA (59).

It has been reported that level of ROS that is present in NMR is no different than that in mouse. Shift from glucose to fructose metabolism is helping NMR in maintaining the antioxidant pool and it is helping NMR from high levels of oxidative stress by ROS (63,64). This gives NMR an upper hand in cancer resistance compared to other rodents.

Materials and Methods

Data collection

- Human oncogene list was downloaded from oncogene database (http://ongene.bioinfo-minzhao.org/download.html) and human TSG list was retrieved from Tumor Suppressor Gene database (https://bioinfo.uth.edu/TSGene/download.cgi).
- Gene positions of naked mole rat can be obtained from UCSC table browser (https://genome.ucsc.edu/cgi-bin/hgTables). Guinea pig, Human, Mouse orthologous gene information was retrieved from http://www.naked-mole-rat.org/.
- UCSC genome browser (https://genome.ucsc.edu/) is used for visualization of shift in CCR cluster across.

Gene functional annotation

Unique genes from the clusters in the distance plot (Figure: 6, Figure: 8a) were filtered out and Toppgene (https://toppgene.cchmc.org/enrichment.jsp) was used for functional annotation.

Data visualization

- Lastz (Penn State) package was used for mapping NMR scaffold to mouse genome
- R software was used for plotting (80).

Results and Observations

In a recent paper published in Molecular Biology and Evolution, it was reported that oncogene without a neighboring Tumor suppressor genes (TSGs) are more prone to amplification (61). Since NMR is very less prone to cancer, we hypothesized that the mean distance between oncogene and TSG in NMR will be less than that in human. So we decided to check whether this is true in NMR or not.

Oncogene – TSG distance

To quantify the distance between oncogenes and neighboring tumor suppressor genes (TSG) in naked mole rat and human, we calculated the position of all oncogenes and TSGs and made a list of nearest TSG to each oncogene.

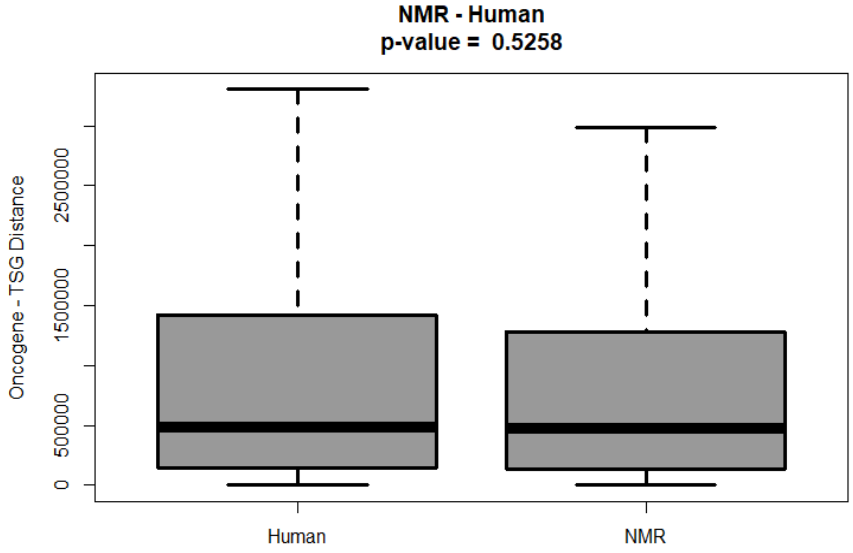

Figure: 5

Distance between oncogenes and TSGs in human and NMR. ($p < .05$)

When plotted, contrary to our hypothesis, we couldn't see any significant change in distance between oncogene and TSG in human and NMR (Figure: 5, $p = 0.5258$)

All gene pair distance

Rejection of our first hypothesis led us to look for all gene pairs rather than just oncogene - TSG pairs. So, we took TSS of all genes and then we calculated distance between all possible gene pairs where both genes come from the same chromosome (in case of human) or in the same scaffold (in NMR) (Figure: 6).

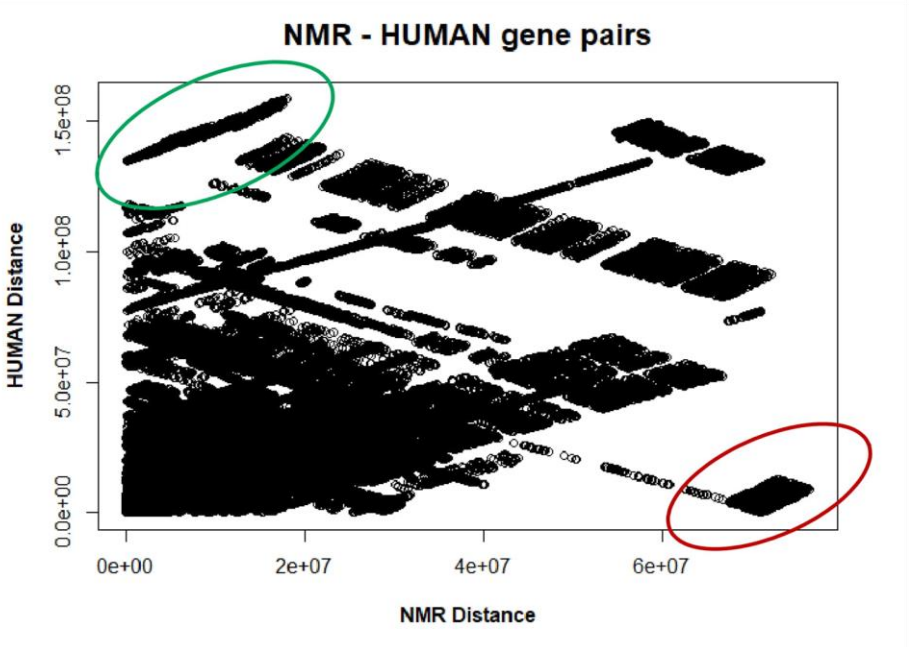

Figure: 6

Scatter plot of gene pair distances showed two clusters, one close to x-axis and other close to y-axis. All the main structures in the figure have come from chromosome 3 of human and JH602043 scaffold of NMR. (Size in bp)

Apart from the beautiful patterns arisen, we found two interesting gene pair cluster in the scatter plot. Each point in the plot represents gene pair with distance in NMR in x-axis and distance in human in y-axis. In the red cluster near to x-axis, each point represents a gene pair where distance in NMR is much higher than that in humans. At the same time, gene pairs in the green cluster, distance in human is greater than that of NMR. The gene cluster that is closer in human, but far in NMR are enriched in hyaluronidase genes (HYAL1, HYAL2, HYAL3) which is highlighted in red. Since NMR cancer resistance is attributed to the presence of HMW-HA, the enrichment of hyaluronidase in the analysis is very important. We also observed that these genes were not present in green cluster. We also observed high enrichment of chemokine receptor genes. To understand what all genes are showing relative change in position, we filtered genes in both clusters and came to know that all these genes are from chromosome 3 in human and a particular scaffold (JH602043) in NMR (Supp_figure:1).

Human vs NMR

In order to know what is happening in chromosome 3 of human and scaffold JH602043 of NMR, we plotted transcription start site (TSS) of each orthologous genes in this region (Figure:7a). Green and red region has HYAL and CCR genes respectively.

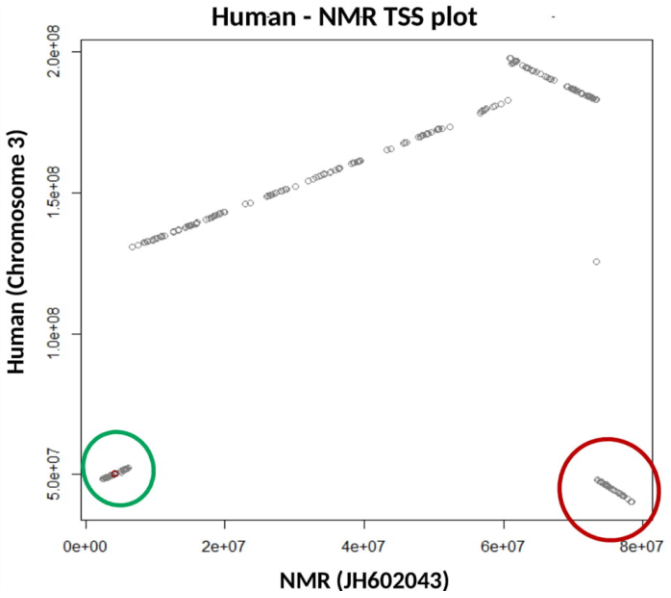

Figure: 7a

TSS plot shows the relative position of HYAL and CCR genes in human and NMR. Huge distance is observed between these cluster in NMR. Green circle contains HYAL genes where as red circle contains CCR genes. Inversion towards the end of NMR scaffold is also evident.

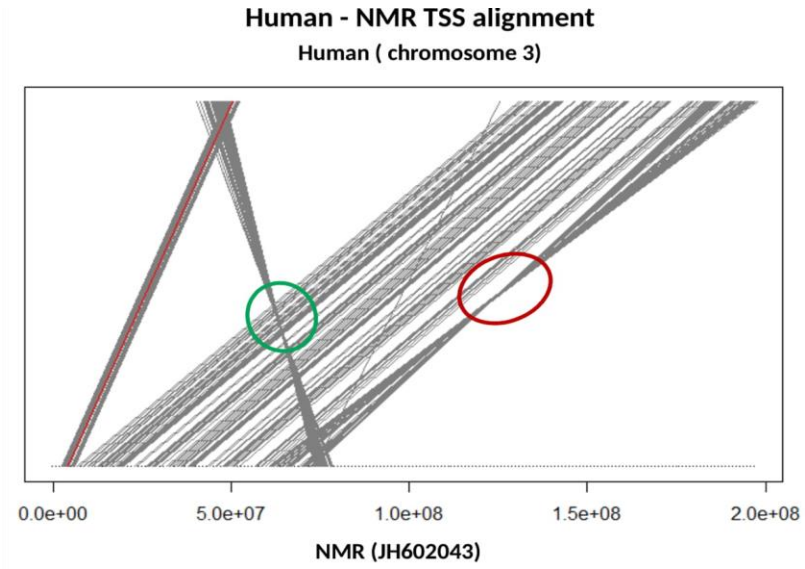

Figure: 7b

Alignment of TSS of genes in NMR and human. Green and red regions show chromosomal inversions. Red line represents the HYAL genes. CCR genes are present in cluster circled in green. X axis represents the distance between gene pairs in NMR scaffold JH602043. Y axis shows the distance between gene pairs in mouse chromosome 9.

We observed two inversions happened toward the end of NMR scaffold (shown in red circle). Chemokine receptor gene cluster (CCR) is the one among two regions that inversion is happened (Green). To get a clear picture, alignment of TSS of genes in human chromosome 3 and NMR scaffold was done (Figure:7b). Genes in red circle and CCR genes are converging whereas HYAL and CCR are diverging. Apart from the inversions, we also found that there is a long-range genome rearrangement happened in NMR.

Two sets of gene clusters (HYAL and CCR) which are closer (~ 3-4 MB) in human is very far (~70 MB) in NMR (Figure: 7b). Genes that were very far apart in human is coming closer to CCR cluster in NMR

Guinea pig vs NMR

Since guinea pig (GP) is closer to NMR in evolutionary tree (Figure: 9), we looked at how these gene clusters are positioned. We observed that CCR and HYAL gene clusters are in the different scaffold (CCR – scaffold 7, HYAL – scaffold 8) in GP. By trying all possible arrangement between these scaffolds, we concluded that no matter what the orientation of these scaffolds in GP's genome, CCR and HYAL clusters will be at least 40 MB apart. We aligned GP scaffolds to Human (Supp_figure: 2a) and similar inversions were observed. When GP scaffolds were compared to NMR (Supp_figure: 2b), no gene rearrangements were observed. The long-range rearrangement in GP is similar to that in NMR. Since GP shows some kind of cancer resistance (62), we hypothesized that shift in CCR cluster might have some implication in cancer.

A smaller shift in Mouse

We went further and checked the gene arrangement in mouse compared to NMR. When scatter plot of gene distances was made, only one gene pair cluster was clearly observed where distance in more in NMR compared to mouse. This cluster consists of genes from Mouse chromosome 9 and same NMR scaffold JH602043 and enriched with both HYAL and CCR. The number of gene pairs that are closer in NMR but far apart in mouse is very less. (Figure: 8a).

Aligning Mouse chromosome 9 and NMR scaffold showed that the shift in CCR cluster in mouse is less. (Figure: 8b). But compared to the distance in human (~ 3-4 MB), distance is mouse is significantly large (~16 MB).

We found that one more segment that is distant to CCR cluster in mouse is joining the CCR cluster in NMR. Inversion is happened in CCR cluster just like in Figure: 7b. Since mouse is not known for cancer resistance, our hypothesis that 'more the shift - less prone to cancer' still holds.

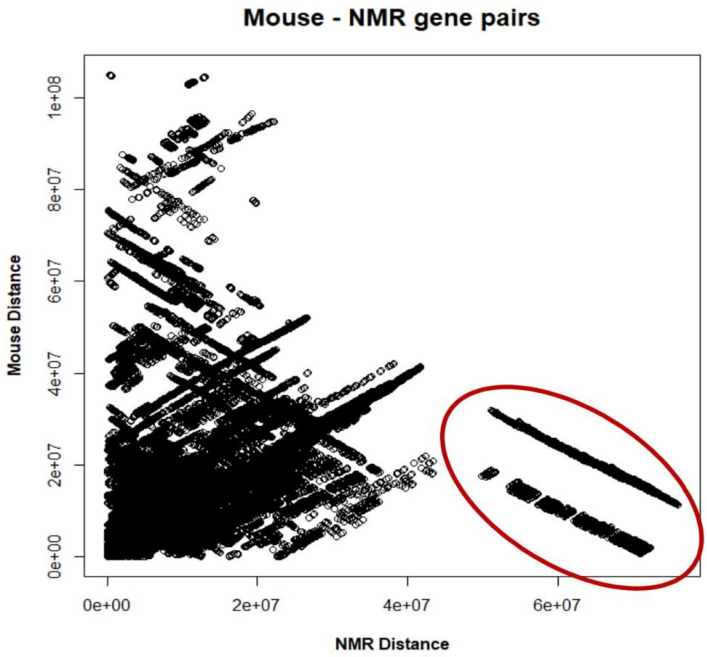

Figure: 8a

Gene pair scatter plot shows HYAL and CCR enrichment (red). X-axis represents the distance between gene pairs in NMR scaffold JH602043. Y-axis shows the distance between gene pairs in mouse chromosome 9.

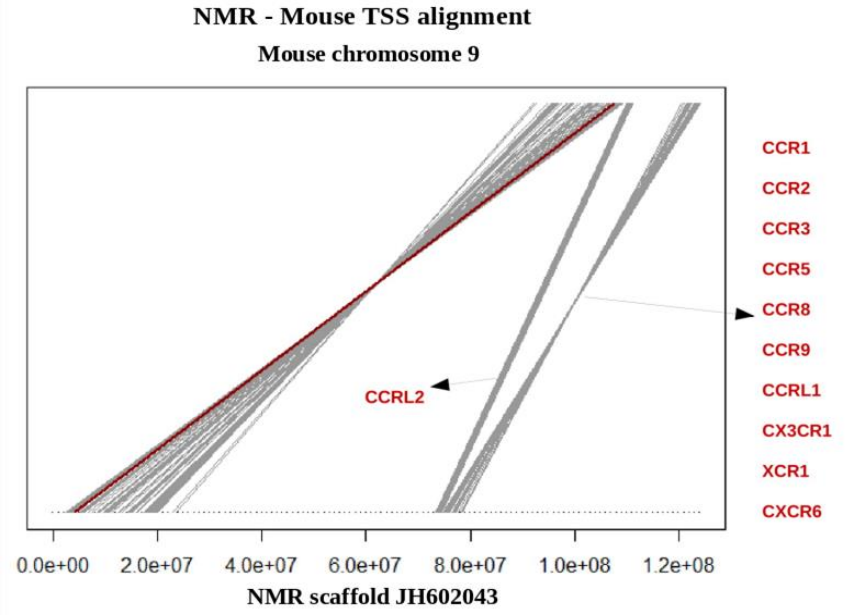

Figure: 8b

TSS alignment of NMR scaffold JH602043 and mouse chromosome 9. NMR has gained CCRL2 in the CCR cluster which was not in mouse CCR cluster.

The rearrangement between CCR and HYAL clusters in rodents

Using the existing available data from UCSC genome browser, we compared the shift in CCR cluster from HYAL cluster in all mammalian lineage. To our surprise, the shift happening in CCR cluster, if at all is happening, is in the rodent lineage (Figure: 9). In other species, the distance between two clusters is almost the same (~3-4 MB) and is not changed much in the course of evolution. The shift is happened in CCR genes, and chemokine signaling is crucial in immune response, cancer progression and aging. Since many species in rodent shows extreme properties in terms of cancer, aging and immunity, we suspect that this rodent lineage specific shift can answer those behaviors in such animals

Due to lesser availability of data for entire rodents, we cannot claim that the shift is happening in all the rodents. But there is a high possibility that this shift will be more evident in other rodents as well. In case of rat and mouse or NMR or GP, the shift in CCR cluster is appeared more to be sub lineage specific manner.

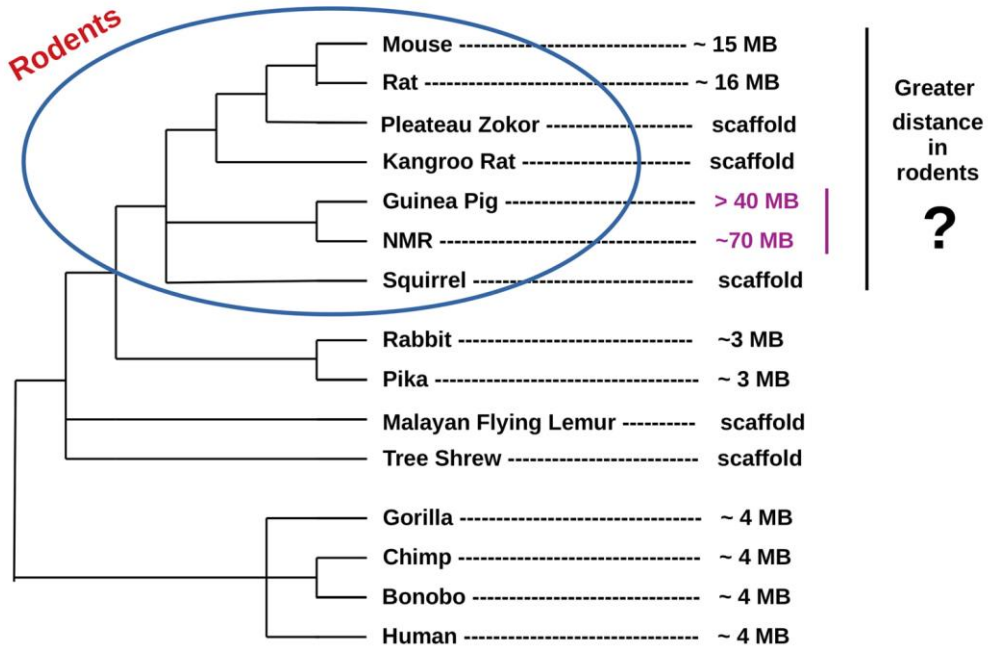

Figure: 9

Distance between CCR and HYAL cluster is shown. Rodent-specific increase in distance is encircled in blue color.

NMR scaffold

The distance between CCR and HYAL cluster in human is very less than that in NMR. HYAL genes and CCR genes have common transcription factor binding site for NfkB (76,77,78,79). It is also observed that both HYAL and CCR clusters are interacting in human. Together we hypothesize that, in humans and other species where the clusters are closer, due to colocalization of clusters, genes involved in these clusters might be coregulating via NFkB pathway. Due to rearrangement of clusters, we can say that the colocalization and co-expression of clusters in NMR is less likely to happen.

To understand what is happening to the NMR scaffold - JH602043, we looked for GC content. We observed high GC content near HYAL and CCR clusters. Very high AT-rich segment is present in between the clusters (figure: 10). Usually the region with high GC content correlates with higher transcription rate (65). The region between CCR and HYAL cluster in mouse and human have more GC content than in NMR.

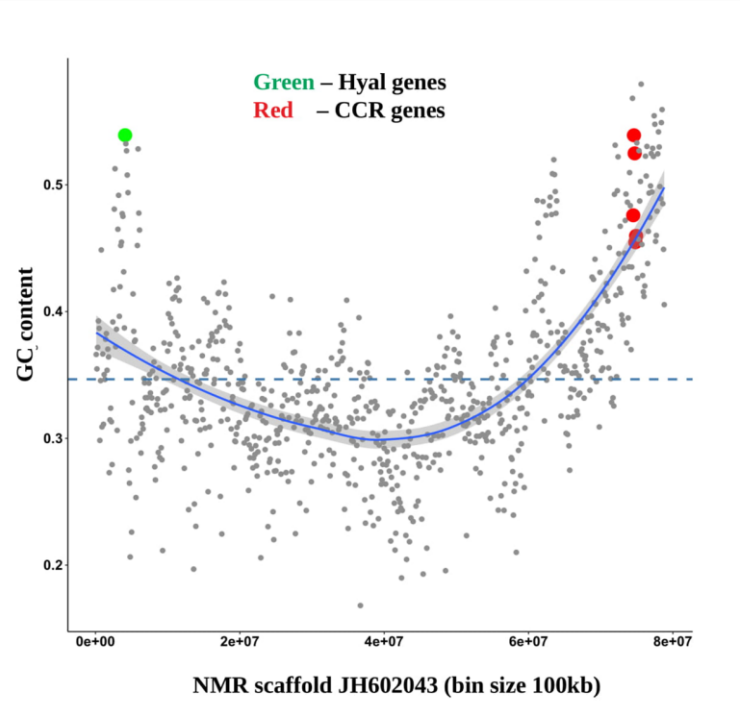

Figure: 10

GC content in NMR scaffold JH602043. Bins are of 100Kb size. Green dot represents the HYAL genes and red dots represent CCR genes. The dotted line represents the mean GC content of the scaffold.

AT-rich domain might have incorporated in between these clusters, predispose the whole region to heterchormatization, or can drag the locus to the nuclear periphery. In case of heterochromatin formation between the cluster, due to leakage, the nearby region can also get suppressed. Nuclear periphery localization will result in association of the domain with nuclear lamin and thus less accessible to transcription. Thus, AT- rich segment insertion can lead to loss of crosstalk between clusters (Figure: 11).

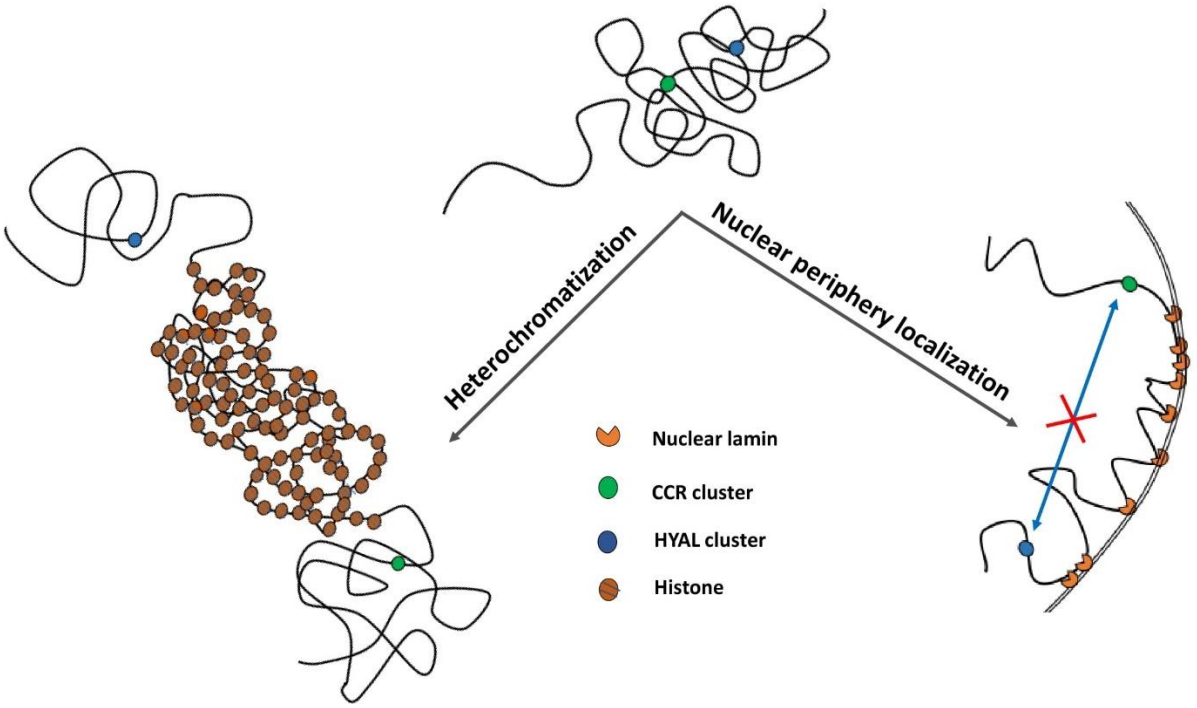

Figure: 11

AT-rich domain insertion can lead to heterochromatization or nuclear periphery localization. This might lead to loss of crosstalk between the clusters or suppression of nearby regions.

To check what might be the case, we mapped the NMR scaffold to mouse chromosomes. We found that most of the in-between region is mapped to only three chromosome segments in mouse (chr3, chr9. chr16) as shown in Figure: 12.

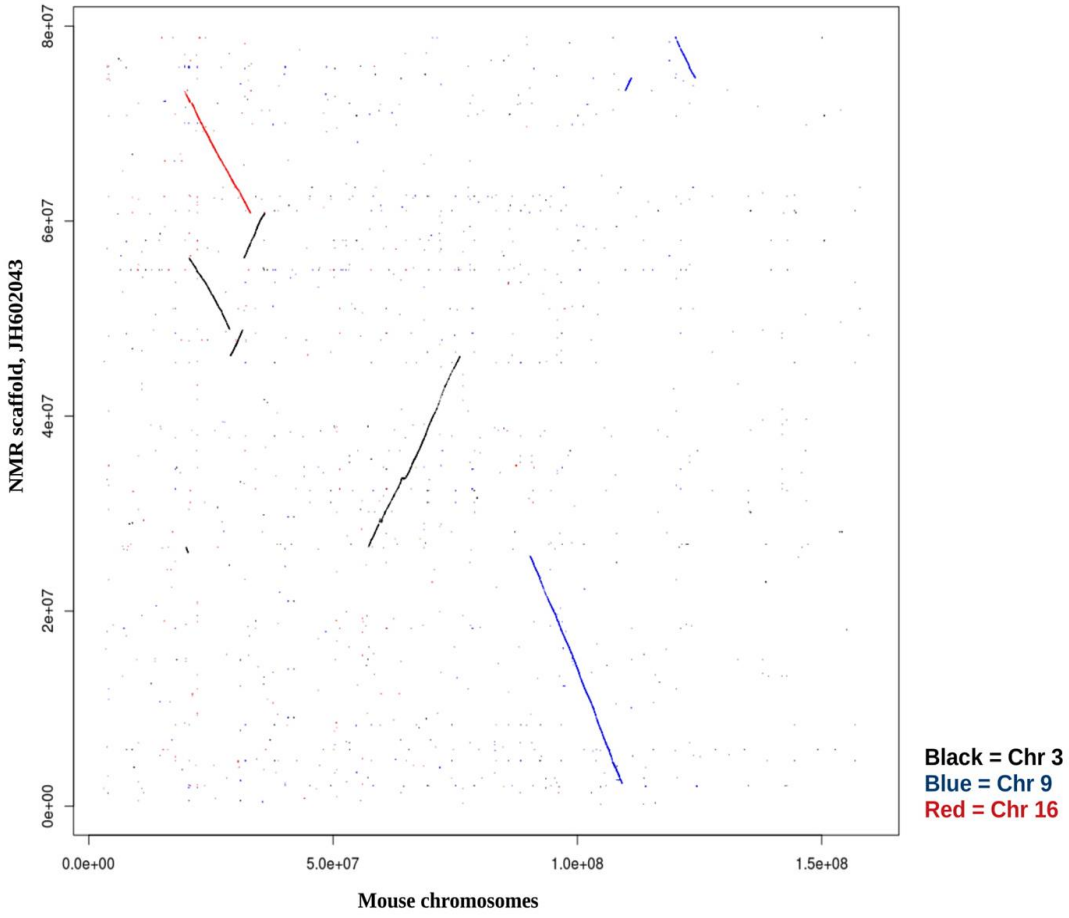

Figure: 12

NMR scaffold JH602043 maps to mouse chromosomes. Most of the in-between region in NMR are mapped to mainly three chromosomes in mouse.

LAD profiling of those segments showed that it is having significant constitutive LAD – constitutive inter LAD ratio (p-value = 2.2e-16) suggesting that the AT- rich segment that inserted between the cluster in NMR might be located in the nuclear periphery with less or no cross-talk between clusters (Figure: 13).

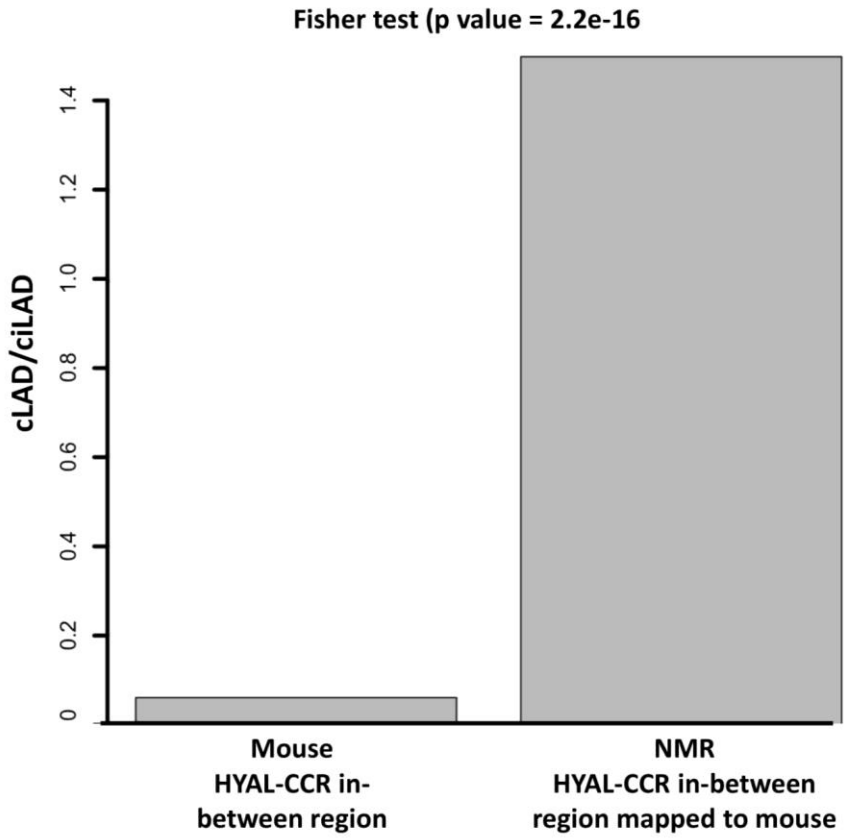

Figure: 13

cLAD/ciLAD is very high for the in-between AT-rich domain. (p value = 2.2e-16) suggesting nuclear periphery localization of the entire domain.

Two inversions shown in figure: 7b are in and around CCR cluster. It is the same region where convergences and divergence happened. This suggests that something is very unusual near CCR cluster.

We observed that genes that are closer to CCR of NMR are enriched with Ubiquitin-like protein-specific endopeptidase activity (SENP2, SENP5), Cysteine-type endopeptidase inhibitor activity (AHSG, HRG, KNG1, FETUB), and SUMO-specific endopeptidase activity (SENP2, SENP5). Protein structural stability and resistance to oxidative stress have a key role to play in longer lifespan of NMR (66). These regions also acquired genes involved in reprogramming (SOX2, KLF4, CMYC) and NMR is reported to have a stable genome that resists reprogramming (71).

And we have found enrichment of CXC-chemokine receptors (CXCR1, CXCR2, CXCR3, CXCR5, CXCR6) near CCR cluster which is not present in mouse or human (Table: 1) and it has been reported that CXC-chemokines have key roles in angiogenesis (74,75). Shift in CXC-chemokine receptors in NMR could have impact on NMR's cancer resistance. In Figure: 8b, we can see CCRL2 chemokine receptor coming to the CCR cluster in NMR. In NMR, more CCR genes are present in the CCR cluster than in mouse or human (Human - 9 CCRs, Mouse - 10 CCRs, NMR - 13 CCRs) and if the shift in CCR cluster has something to do with expression levels, this may have a direct correlation with NMR cancer resistance.

Human	CXCR6, CCR1, CCR2, CCR3, CCR5, CCR9, CCRL1, CCRL2, XCR1
Mouse	CXCR6, CCR1 CCR2, CCR3, CCR5, CCR8, CCR9, CCRL1, CXCR1, XCR1
NMR	CXCR1, CXCR2, CXCR3, CXCR5, CXCR6, CCR1, CCR2, CCR3, CCR5, CCR6, CCR8, CCR9, CCRL2

Table: 1

Chemokine receptors in the CCR cluster in human, mouse and NMR.

In NMR few pro-inflammatory CXC-chemokines (CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8) are present near (2-2.5 MB) HYAL cluster. AT-rich domain insertion in NMR might have suppressed the nearby region including the expression of CXC-chemokines.

Conclusions

So far, we have managed to find the rearrangement between CCR and HYAL clusters which happens exclusively in rodents. Within rodents, the distance varies greatly. The organism that shows more cancer resistance is having huge shift in CCR cluster. We also observed that the distance is increasing in a sub-lineage specific manner (NMR and GP, Mouse and Rat) suggesting the shift might have happened in the course of evolution. More shift is shown by organisms that didn't undergo many changes in the evolutionary history.

Genes in both clusters have common transcriptional regulator, NFkB suggests that possible co-regulation at transcription level by colocalization. Colocalization of CCR and HYAL cluster is less probable in NMR due to the increased distance between clusters.

Most of CCR genes in our cluster are inflammatory in nature. And if this cluster is repressed, only inflammatory process is affected not the homeostasis. We also found that the nearby region of CCR cluster in NMR have gained genes associated with protein degradation and reprogramming. The region near CCR cluster is showing drastic rearrangement including two inversions. All these together suggests that CCR cluster in NMR could have major role in cancer and aging. This also suggests that there is an evolutionary position effect playing in the background regulating expression of the locus and thus cancer resistance, immunity and aging.

Chromatin undergoes dynamic, organizational changes over an organism's life and may be a contributing cause of aging. Aging also involves smoothing of the existing epigenetic patterns and loss of heterochromatin. Epigenetic regulation has been proposed to be an important player in aging and cancer (67,68,69,70). In NMR presence of AT-rich domain in-between the clusters might predispose the whole region to heterochormatize or can drag the locus to the nuclear periphery. This will lead to less transcription of the genes in the cluster. Since the genes involved in and around the clusters are having a crucial role in cancer and aging, this might explain NMRs stable epigenome, longer lifespan and its cancer resistance.

More focus should be given to 3D genome organization as reprogramming genes are also involved in our region of interest. Thus inflammation, cancer and aging should be seen together through a 3D organization and oxidative point of view. Depending on epigenome marks on CCR cluster region, the expression levels of chemokine receptors changes and thereby, the crosstalk changes and thus immune response and cancer metastasis. Reactive Oxygen Species mediated aging is also linked via hyaluronan degradation and chemokine receptor expression. We further hypothesize that nuclear periphery localization and chromatin structure of CCR cluster might depend up on the physiology and habitat of rodents.

Future Prospects

- CCR receptor expression varies a lot with the TME and the cells involved shows varying expression depending on the involvement in the inflammation response. To get a complete picture, we must look at the chromatin state, nuclear localization, expression pattern and epigenetic marks of all genes involved (HYALs, CCRs, chemokines, reprogramming genes) in different cell types (fibroblast, different types of immune cells, epithelial cells, etc..) in different types cancer and during different stages of life.
- Since Blind Mole Rat (BMR) also shows amazing cancer resistance and higher levels of HWM-HA, comparing genome organization of NMR and BMR will be helpful
- Relation between oxidative stress and chromosome rearrangements.
- Facilitative heterochromatin possibilities in CCR region under stress condition
- Figuring out possible reasons for genomic rearrangement of this extend?

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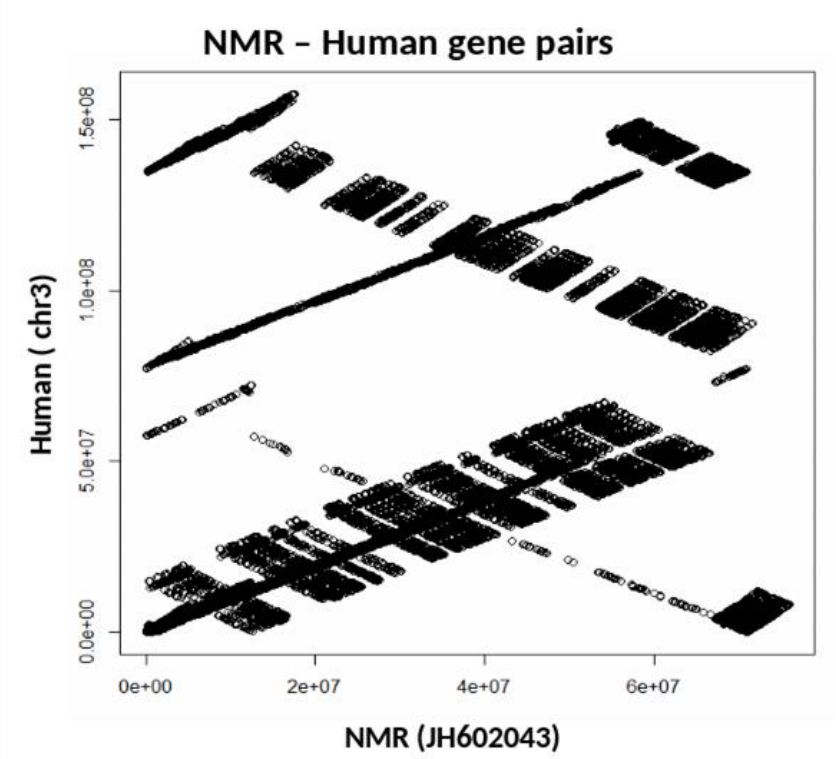
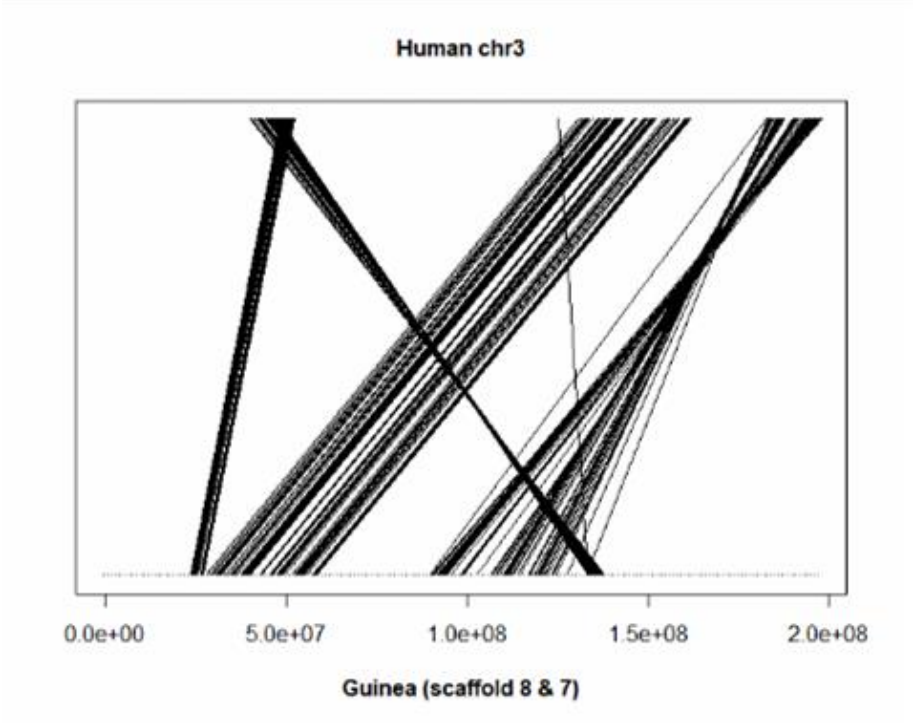
Supplementary figures



Supp_figure: 1

Scatter plot of Human chr 3 and NMR scaffold JH602043 gene pairs. Most of the structures are coming from these regions.



Supp_figure: 2a

Scaffolds (7 & 8) are aligned to human chromosome 3. Guinea pig shows similar gene arrangement as in NMR

Supp_figure: 2b

Guinea pig scaffold – NMR JH602043 TSS alignment shows guinea pig is no much different than NMR