Genome Editing and Engineering
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Module - 09
Genome Engineered Disease Modelling
Lecture - 01
Cancer Disease Models - Part B

Welcome to my course on Genome Editing and Engineering. We are discussing module 9 Genome Engineered Disease Modelling and we have been discussing about Cancer Disease Models and we continue our discussion in Part B of this lecture.

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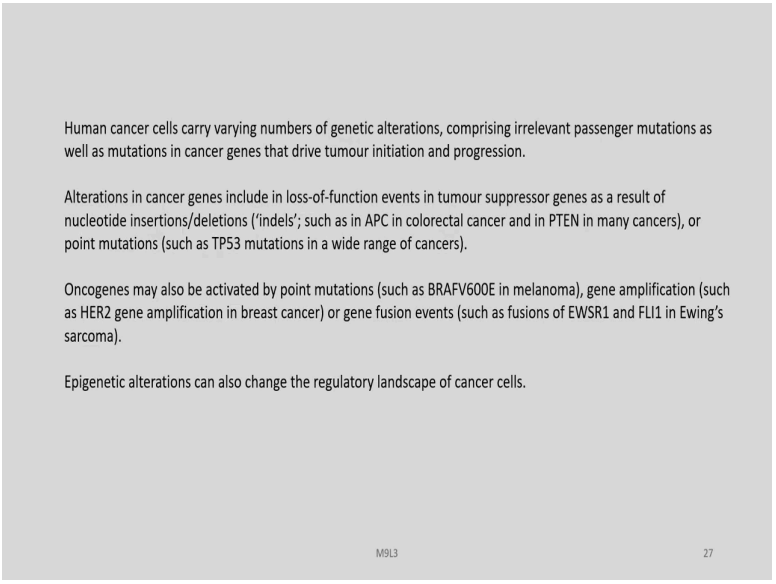

We have learnt in the last unit that human cancer cells carry varying numbers of genetic alterations comprising irrelevant passenger mutations as well as mutations in cancer genes that drive tumour initiation and progression.

Alteration in cancer genes include loss of function events in tumour suppression genes as a result of nucleotide insertion deletions. Indels such as in APC in colorectal cancer and in PTEN in many cancers or point mutation such as TP53 mutations in a wide range of cancers. Oncogenes may also be activated by point mutations such as BRAFV600E in melanoma. Gene and amplification such as HER2 gene amplification is breast cancer or gene diffusion event such as fusions of EWSR1 and FLI1 in Ewing's sarcoma.

Epigenetic alterations can also change the regulatory landscape of cancer cells. And in the modelling of a cancer we have to imitate all these kind of genetic changes and built up these regulatory landscape of the genes and genetic elements inside in the cancer cells.

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In this context CRISPR Cas9 has been an invaluable addition to the genetics toolkit to allow generation of mouse models that faithfully recapitulate the myriad of genetic and epigenetic modifications seen in human cancers.

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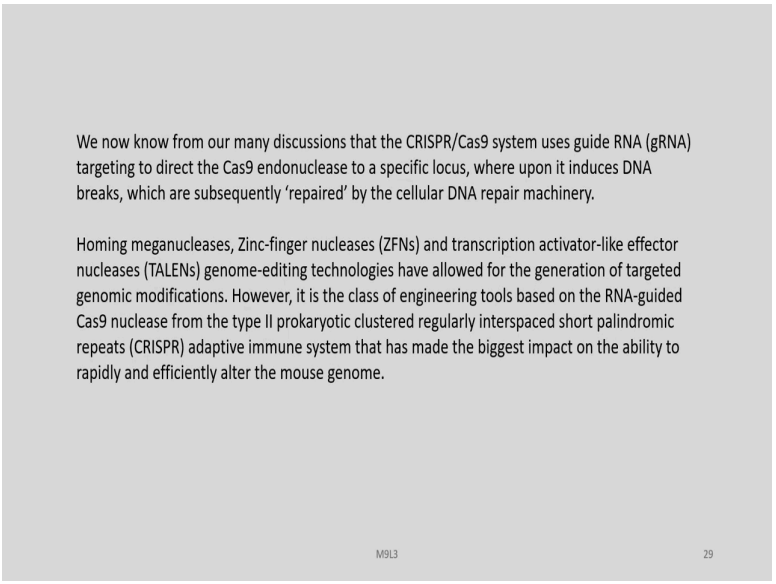

From our innumerable discussions in the past, we now know that CRISPR Cas9 system uses guide RNA targeting to direct the Cas9 endonuclease to a specific locus where upon it induces DNA breaks which are subsequently repaired by the cellular DNA repair mechanism machinery.

Homing meganucleases ZFNs and TALENs genome editing technologies have allowed for a generation of targeted genomic modifications. However, it is the class of engineering tools based on the RNA-guided Cas9 nuclease from the type II prokaryotic clustered regularly interspaced short palindromic repeats CRISPR adaptive immune system that has made the biggest impact on the ability to rapidly and efficiently alter the mouse genome.

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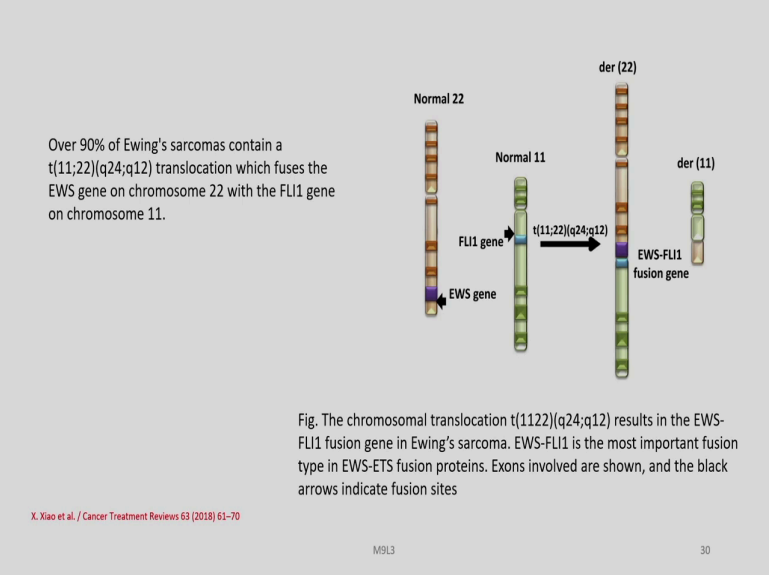

We have discussed about the Ewing's sarcoma in the earlier part of this lecture and there we have discussed about the use of ZFN in generating a translocation which is found in the diseased case.

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CRISPR Cas9 has also been efficiently used for doing the similar kind of modelling by Torres and his colleagues in certain human cancers chromosomal translocations are generated through the illegitimate joining of two non homologous chromosomes affected by double strand breaks. Reliable methodologies to reproduce precise reciprocal tumour associated chromosomal translocations are required to gain insight into the initiation of leukaemia and sarcomas.

The methodology developed by Torres et al, for generating cancer related human chromosomal translocations in vitro based on the ability of the RNA guided CRISPR Cas9 system induce DSB at the defined positions.

Through these they produced human cell lines and primary cells bearing chromosomal translocations resembling those described in acute myeloid leukaemia and Ewing's sarcoma at very high frequencies through fish and molecular analysis at the mRNA and protein levels of the fusion genes involved in these engineered cells. They found CRISPR Cas9 to be highly reliable accurate and a powerful tool for cancer modelling studies.

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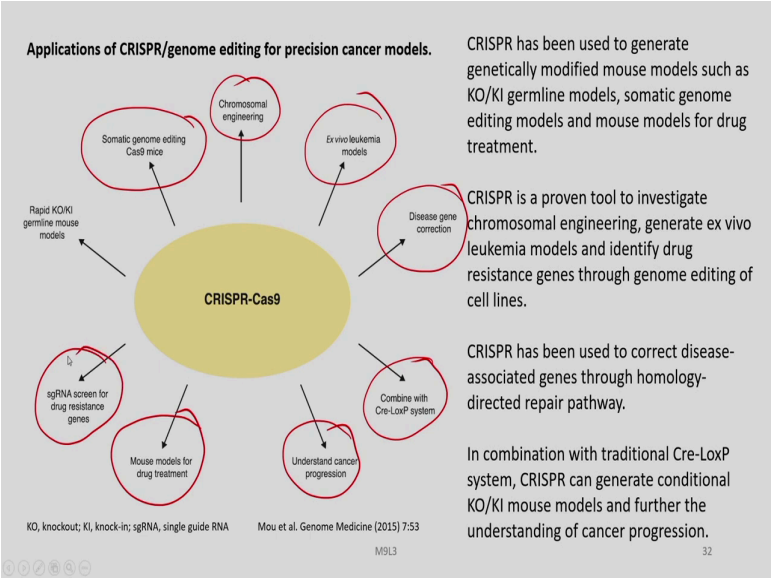

So, CRISPR Cas9 can have huge impact in cancer research. The various applications of CRISPR genome editing for precision cancer models can be seen in this figure. So, it is used for the rapid knockout knock in germline mouse model generation. Somatic genome editing a Cas9 mice then for chromosomal engineering ex vivo leukemia models development, disease gene correction combine with Cre-LoxP system and for understanding cancer progression and developing mouse models for drug testing and treatment, then sgRNA screen for drug resistant genes.

CRISPR has been used to generate all these kind of studies and it is a proven tool to investigate chromosomal engineering, generate ex vivo leukemia models and identify drug resistance genes through genome editing in cell lines. It can be used to correct disease associated genes through homology directed repair pathway and in combination with the traditional Cre-LoxP system, CRISPR can generate conditional knockout knock in mouse models and further the understanding of cancer progression.

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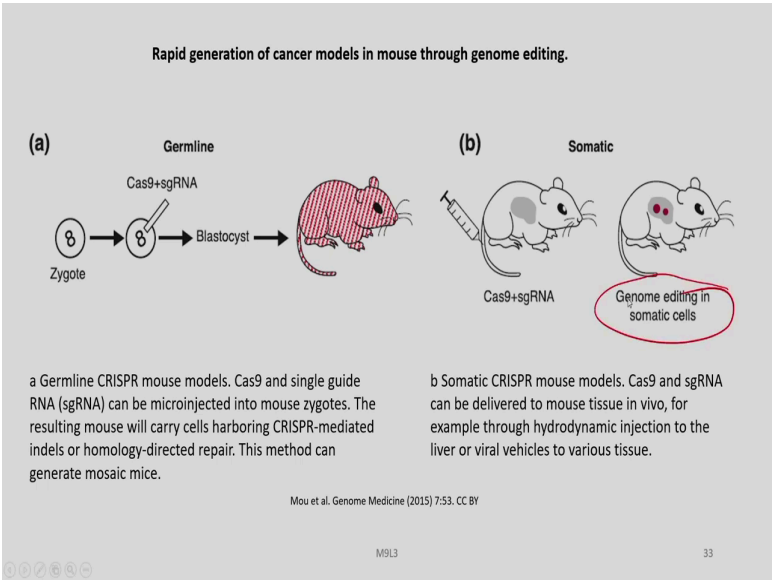

Rapid generation of cancer models in mouse through genome editing is possible using CRISPR Cas9. So, here in this figure a, you can see the production of germline CRISPR mouse models. Cas9 and single guide RNA can be micro injected into mouse zygotes. The resulting mouse will carry cells harboring CRISPR mediated indels or homology directed repair and this method generates mosaic mice. In the case of figure b you can see the somatic CRISPR mouse models production Cas9 and sgRNA can be delivered to mouse tissue in vivo.

For example through hydro dynamic injection to the liver or viral vehicles to various tissues and we obtain genome editing in the somatic cells as a outcome of this procedure.

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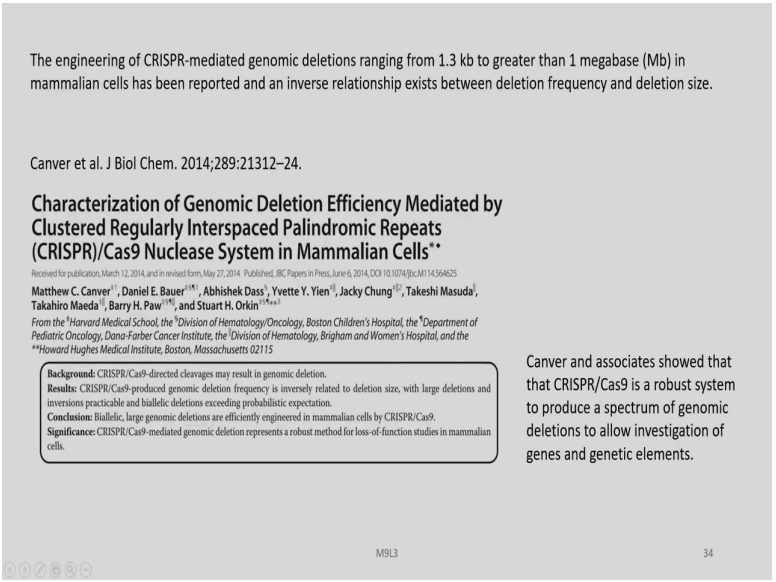

The engineering of CRISPR mediated genomic deletions ranging from 1.3 kb to greater than 1 mega bases in mammalian cells has been reported and an inverse relationship exists between deletion frequency and deletion size.

This work by Canver et al and in journal of Biological Chemistry on the characterization of genomic deletion efficiency mediated by CRISPR Cas9 nuclease system in mammalian cells is considered as a very important study in this regard and they showed that CRISPR Cas9 is a robust system to produce a spectrum of genomic deletions to allow investigations of genes and a genetic elements.

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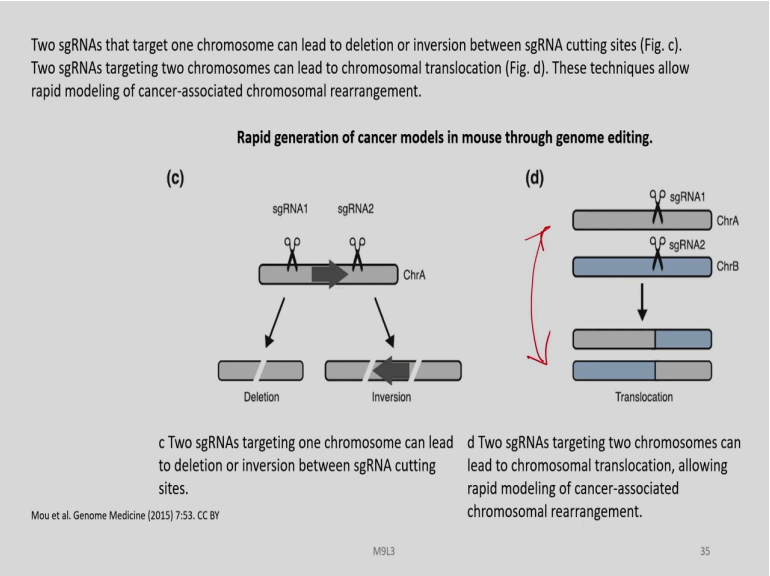

Two sgRNAs that target one chromosome can lead to deletion or inversion between sgRNA cutting sites as seen in this figure c. Two sgRNAs targeting two different chromosomes can lead to a chromosomal translocation as shown in the figure d. These techniques allow rapid modelling of cancer associated chromosomal rearrangement and we have seen in the case of Ewing's sarcoma, how these translocations are utilized.

So, in figure c you can see two sgRNAs are targeting one chromosome due to which there is either a gap or deletion or this is getting inverted and while in figure d there is a occurrence of translocation.

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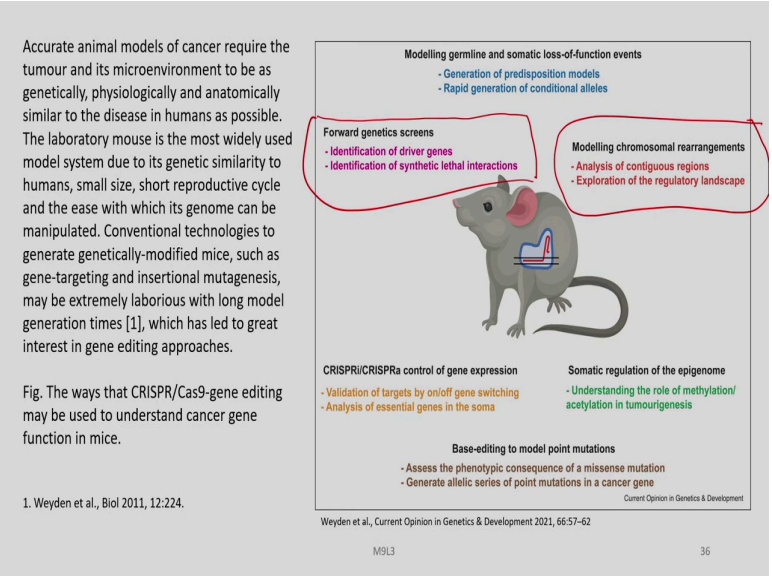

Accurate animal models of cancer require the tumour and its micro environment to be as genetically physiologically and anatomically similar to the disease in humans as possible.

And while studying the cyclic process in cancer modelling there is a step of a model refinement or model improvement. So, we may not be able to build up the perfect model in the first circle, but after learning about the various problems and false fallacies we can improve upon the model and make them as much similar to the disease in humans and generate a real model.

The laboratory mouse is the most widely used model system due to its genetic similarity to humans. Small size short reproductive cycle which we have already discussed. And above all the ease with which its genome can be manipulated convention and technologies to generate genetically modified mice such gene targeting and insertional and mutagenesis may be extremely laborious with long model generation times which has led to great interest in gene editing approaches. In this figure we can see the various ways CRISPR Cas9 gene editing is used to understand cancer gene function in mice.

The modelling germline and somatic loss of function events for the generation of predisposition model, rapid generation of conditional alleles, we may have forward genetic screen identification of driver genes and identification of synthetic lethal interactions. In a modelling of chromosomal rearrangements the analysis of contiguous regions and exploration of the regulatory landscape is involved. In the case of CRISPRI and CRISPRa control of gene

expression is possible. Validation of targets by on off gene switching analysis of essential genes in the body or soma.

Somatic regulation of the epigenome for understanding the role of methylation acetylation in tumour genesis. We may also use the base editing to model point mutations here we have access to the phenotypic consequences of a missense mutation and generate allelic series of point mutations in a cancer gene.

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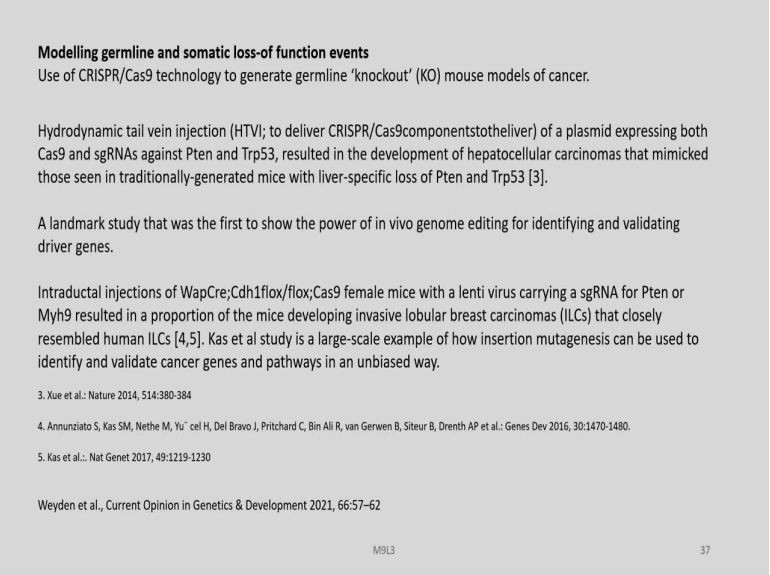

Let us now discuss these various aspects of CRISPR Cas9 gene editing in the generation of cancer models one by one. Let us start with the modelling of germline and somatic loss of function events. CRISPR Cas9 technology can be used to generate germline knockout mouse models of cancer.

Hydrodynamic tail vein injection of a plasmid expressing both Cas9 and sgRNAs against Pten and Trp53 has resulted in the development of hepatocellular carcinoma that mimicked those seen in traditionally generated mice with liver specific loss of Pten and Trp53. This was a landmark study which for the first time showed the power of in vivo genome editing for identifying and validating driver genes.

Intraductal injections of WapCre;Cdh1flox/flox;Cas9 female mice with a lenti virus carrying a sgRNA for Pten or Myh9 resulted in a proportion of the mice developing invasive lobular breast carcinomas that closely resemble human ILCs

Kas et al studied a large scale example of how insertion mutagenesis can be used to identify and validate cancer genes and pathways in an unbiased way.

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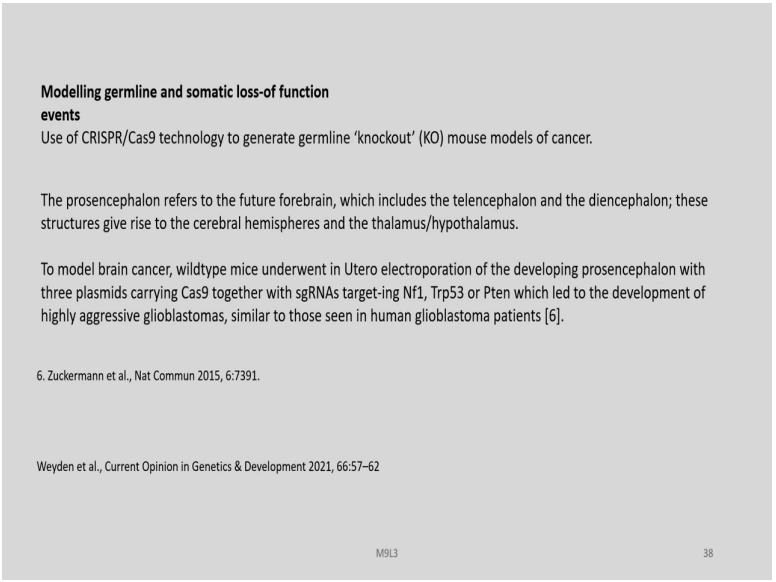

The prosencephalon refers to the future forebrain which includes the telencephalon and the diencephalon; these structures give rise to the cerebral hemisphere and the thalamus or hypothalamus.

For modelling brain cancer wild type mouse underwent in Utero electroporation of the developing prosencephalon with three plasmids carrying Cas9 together with sgRNAs targeting Nf1, Trp53 or Pten which led to the development of highly aggressive glioblastomas similar to those seen in human glioblastoma patients.

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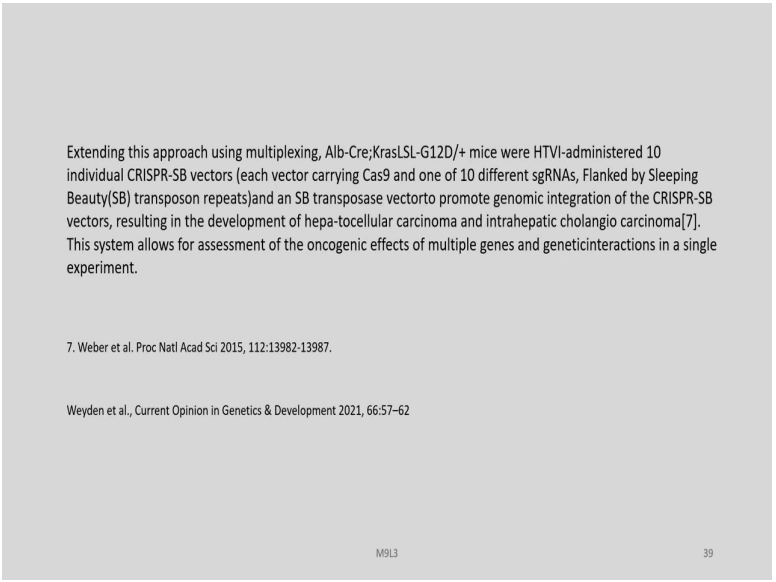

Extending this approach using multiplexing Alb-Cre;KrasLSL-G12D oblique positive mice where HTV administered as 10 individual CRISPR-SB vectors. Each vector carrying Cas9 and one of the 10 different sgRNAs is flanked by sleeping beauty transposon repeats and an SB transposase vector to promote genomic integration of the CRISPR-SB vectors resulting in the development of hepatocellular carcinoma and intra hepatic cholangio carcinoma. This system allows for assessment of the oncogenic effects of multiple genes and genetic interactions in a single experiment.

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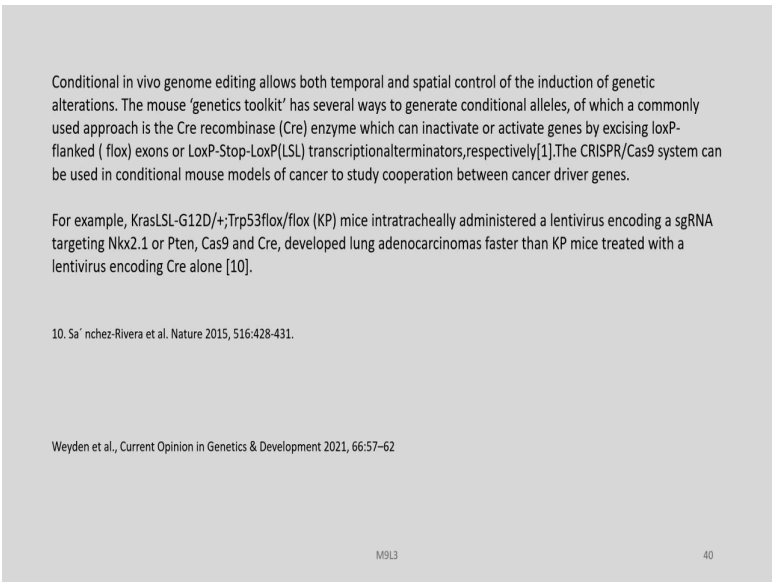

Conditional in vivo genome editing allows both temporal and spatial control of the induction of genetic alterations. The mouse genetics toolkit has several ways to generate conditional alleles of which a commonly used approach is the Cre recombinase Cre enzyme which can inactivate or activate genes by exercising excising LoxP flanked exons or LoxP-Stop-LoxP transcriptional terminators respectively. The CRISPR Cas9 system can be used in conditional mouse models of cancer to study cooperation between cancer driver genes.

Some examples are KrasLSL-G12D/+;Trp53 flox/flox (KP) mice intra tracheally administered a lentivirus encoding a sgRNA targeting Nkx2.1 or Pten Cas9 and Cre developed lung adenocarcinomas faster than KP mice treated with a lentivirus encoding Cre alone.

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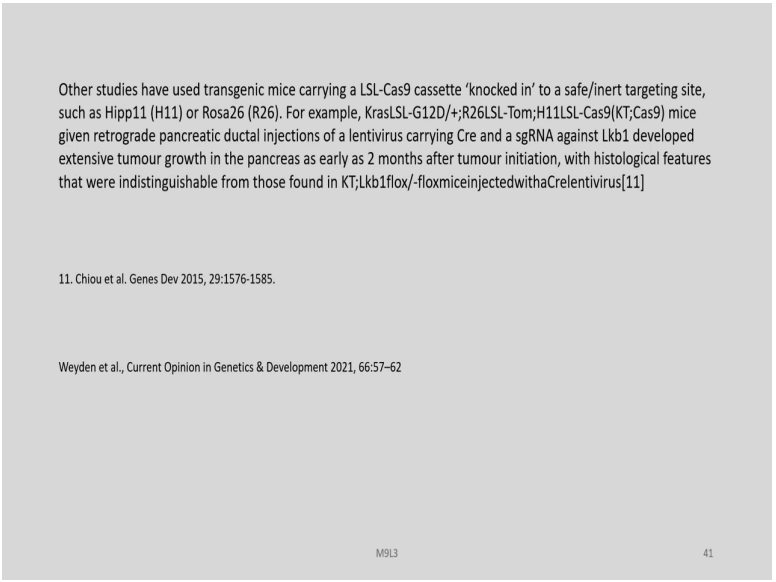

In other studies they have used transgenic mice carrying a LSL Cas9 cassette knocked in to a safe inert targeting site such as Hipp11 or Rosa26 and examples are krasLSL-G12D/+;R26LSL-Tom;H11LSL-Cas9(KT;Cas9) mice given retrograde pancreatic ductal injections of a lentivirus carrying Cre and a sgRNA against Lkb1 developed extensive tumour growth in the pancreas as early as 2 months after tumour initiation, with histological features that were indistinguishable from those found in KT Lkb1 flox or flox mice injected with a Cre lentivirus.

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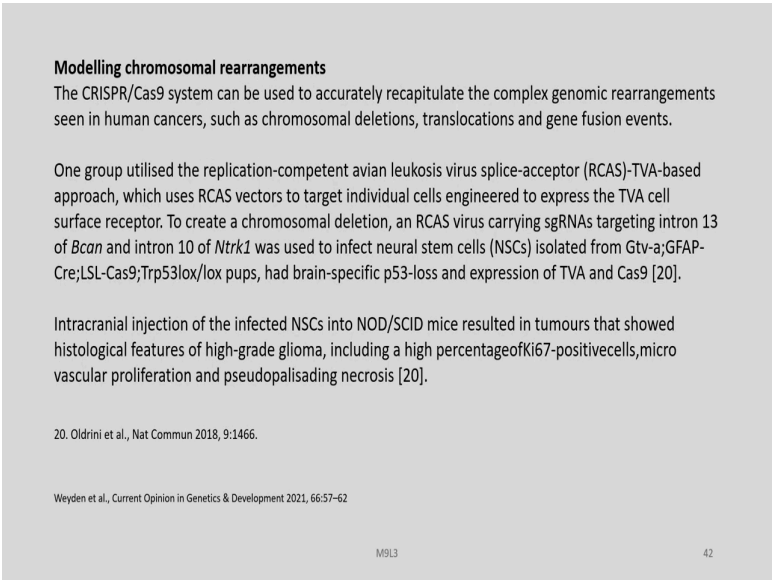

Another application of CRISPR Cas9 is in the modelling chromosomal rearrangements about which we have already discussed in the case of Ewing sarcoma. One group utilized the replication competent avian leukosis virus splice acceptor a based approach which uses RCAS vector to target individual cells engineer to express the TVA cell surface receptor.

To create a chromosomal deletion and RCAS virus carrying sgRNA targeting intron 13 of Bcan and intron 10 of Ntrk1 was used to infect neural stem cells isolated from Gtv-a; GFAP-Cre;LSL-Cas9;Trp53 lox/lox pups, had brain specific p53 loss and expression of TVA and Cas9.

Intracranial injection of the infected NSCs into NOD/SCID mice resulting in tumours that showed histological features of high grade glioma including a high percentage of Ki67 positive cells microvascular proliferation and pseudopalisading necrosis.

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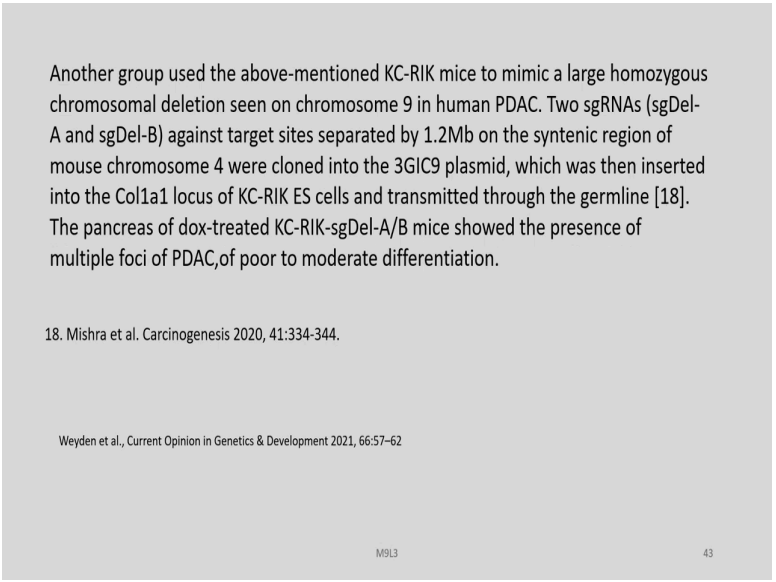

Another group used the above mentioned KC-RIK mice to mimic a large homozygous chromosomal deletion seen on chromosome 9 in human PDAC. Two sgRNAs against target site separated by 1.2 Mb on the syntenic region of mouse chromosome 4 cloned into the 3GIC9 plasmid, which was then inserted into the Col1a1 locus of KC RIK ES cells and transmitted throughout the germline. The pancreas of dox treated KC-RIK-sgDel-A/B mice showed the presence of multiple foci of PDAC of poor to moderate differentiation.

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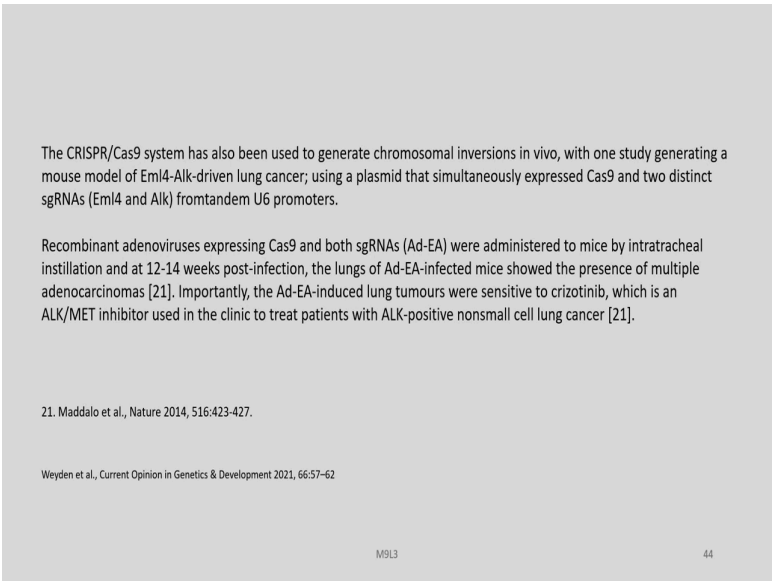

The CRISPR Cas9 system has also been used to generate chromosomal inversions in vivo with one study generating a mouse model of Eml4-Alk driven lung cancer; using a plasmid that simultaneously express Cas9 into distinct sgRNAs from tandem U6 promoters.

Recombinant adenoviruses expressing Cas9 and both sgRNAs were administered to mice by intratracheal installation and at 12 to 14 weeks post infection the lungs of Ad-EA infected mice showed the presence of multiple adenocarcinomas. Importantly the Ad-EA induced lung tumours were sensitive to crizotinib which is an ALK/MET inhibitor used in the clinic to treat patients with ALK-positive non small cell lung cancer.

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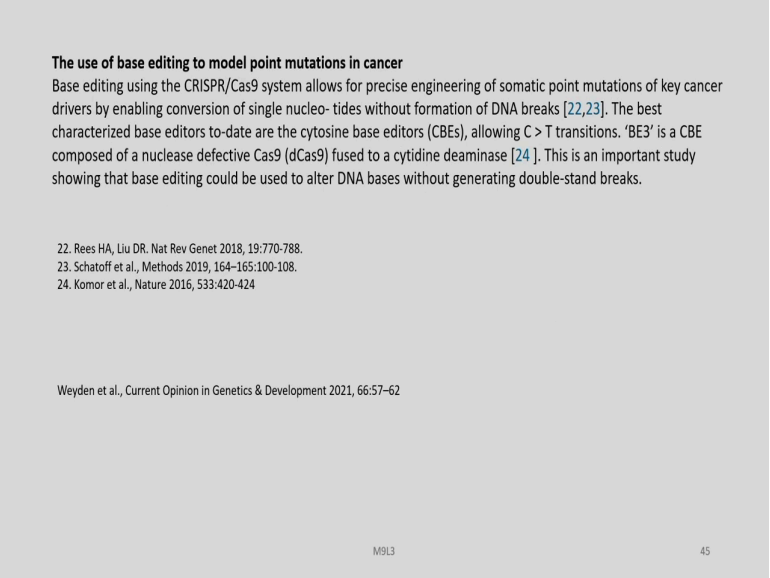

The use of base editing to model point mutation in cancer has also been actively pursued using CRISPR Cas9 system which allows for precise engineering of somatic point mutations of key cancer drivers by enabling conversion of single nucleotides without formation of DNA breaks.

The best characterized base editors to date are the cytosine base editors, allowing C to T transitions. BE3 is a CBE composed of a nuclease defective Cas9 fused to a cytidine deaminase. This is an important study showing that base editing could be used to alter DNA bases without generating double strand breaks.

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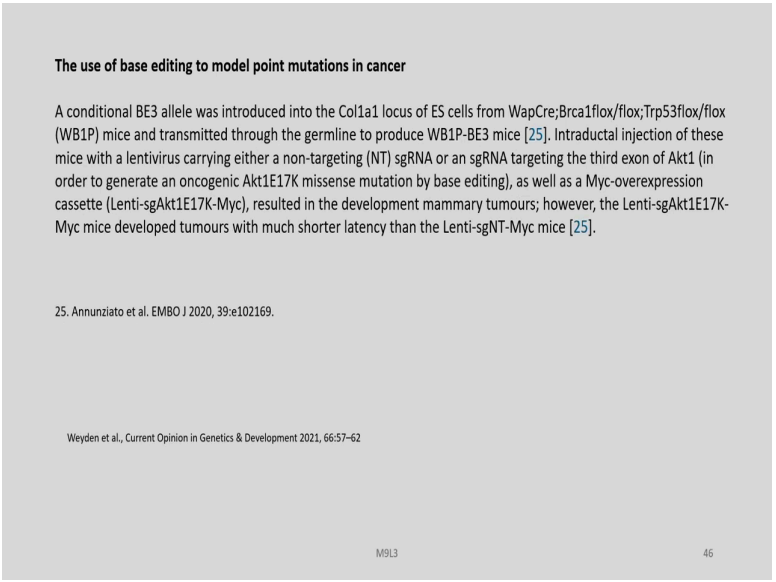

A conditional BE3 allele was introduced into the Col1a1 locus of ES cells from WapCre; Brca1flox/flox;Tr53flox/flox WB1P mice and transmitted through the germline to produce WB1P-BE3 mice. Intraductal injection of these mice with a lentiviral virus carrying either a non targeting sgRNA or an sgRNA targeting the third exon of a Akt1 in order to generate an oncogenic AktE17K missense mutation by base editing as well as by Myc-overexpression cassette resulted in the development of mammary tumours.

However, the Lenti-sgAkt1E17K-Myc mice developed tumours with much shorter latency than the Lenti-sgNT-Myc mice. So, these are some of the achievements of CRISPR Cas9 in the field of base editing to model point mutations in cancer.

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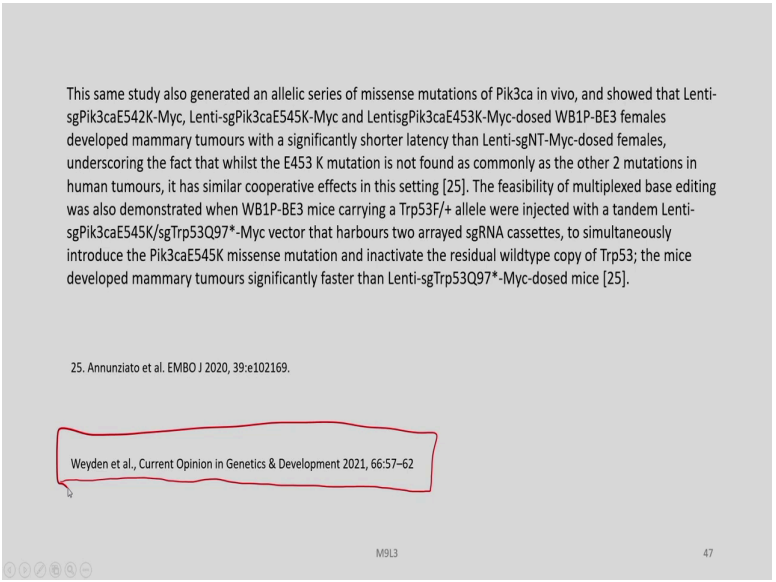

The same study also generated an allelic series of missense mutations in Pik3ca in vivo and showed that Lenti-sgPik3caE542K-Myc, then Lenti-sgPik3caE545K-Myc and Lenti-sgPik3caE453K-Myc dosed WB1P-BE3 females developed mammary tumours with a significantly shorter latency than Lenti-sgNT-Myc dosed females underscoring the fact that whilst the E453 K mutation is not found as commonly as the other two mutations in human tumours, it has similar cooperative effects in this setting. The feasibility of multiplexed base editing was also demonstrated when WB1P-BE3 mice carrying Trp53F/+ allele were injected with a tandem Lenti-sgPik3caE545K/sgTrp53qQ97*-Myc vector that harbours two arrayed sgRNA, to simultaneously introduce the Pik3caE545K missense mutations and inactivate the residual wild type copy of Trp53.

The mice develop mammary tumours significantly faster than Lenti-sgTrp53Q97*-Myc dosed mice. The majority of these studies discussed here has been taken from this review by Weyden et al in Current Opinion in Genetics and Development and the original references are also quoted in exact numbers in the text as references. So, for further details kindly consult the original articles from which these case studies have been collected and compiled by Weyden et al.

Overall to conclude the various gene editing technologies like ZFN Talen and CRISPR Cas9 has been successfully used in creating models for cancer in cells as well as in animals. And this makes the study of cancer the discovery of cancer related genes as well as the

development of anti cancer drugs quite easy than in earlier times. So, genome editing technologies for cancer disease modelling has a very big impact in the development of various cancer based therapeutics. With this we come to the conclusion of module 9.

Thank you for your patient hearing.