Reporting of Mammary Tumours in Sprague Dawley/NIN Mutant (Hairless) Rat Strain.

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Abstract:
A spontaneous genetic mutation in Sprague Dawley rats was detected at the ICMR-NIN animal facility. The spontaneously mutated rat was examined for body composition, serum biochemistry, peripheral blood CD4 and CD8 cell population, and spontaneous tumour growth. We observed the spontaneous development of tumours in 24 animals on crossbreeding. The animals showed no significant differences in vital organ structure as compared to the heterozygous animals. Interestingly, both homozygous (hairless) and heterozygous rats developed spontaneous fibroadenomas in their mammary glands during adulthood, which was distinct from the parent NIN/SD rat colony. Importantly, there was no connection between the biochemical and T cell population and the presence of these tumours. The histological characteristics of the tumours closely resembled mammary tumours in humans. In conclusion, the hairless SD rat model with thymus will serve as a valuable tool in cancer research, particularly in mammary tumour studies.

Keywords: *Hairless rat, Sprague Dawley, mammary tumour*

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Introduction

Globally, breast cancer is on the rise, particularly in Asia, with India facing a substantial impact. In 2017, breast cancer comprised 25% of female cancer cases, with an incidence rate of 25.8 per 100,000 and a mortality rate of 12.7 per 100,000. Alarming trends are evident among younger Indian women (30 to 40 years old), constituting 48% of patients and showing a concerning increase over the past 25 years. The mortality-to-incidence ratio varies between rural and urban registries. The 5-year survival ratio in India from 2000 to 2014 is a dismal 66.1%, the lowest among analysed countries(Madhav, Nayagam, et al. 2018).

Breast cancer stands as a formidable global health challenge(Lu, Steeg, et al. 2009), impacting the lives of millions of individuals worldwide(Beral, Banks, et al. 2003, Tao, Shi, et al. 2015, Park, Han, et al. 2021). Numerous dietary components have undergone investigation regarding their potential influence on both the risk and management of breast cancer. Foods rich in antioxidants, such as fruits and vegetables, have displayed an association with lowered risk due to their capacity to counteract oxidative stress(Li, Ambrosone, et al. 2009) and inflammation(Choudhury, Kandimalla, et al. 2018) —two factors intricately tied to the development of cancer(Kang 2002, Shivappa, Blair et al. 2017). Additionally, dietary fibre sourced from whole grains, legumes, and vegetables not only aids in digestion but also possesses the potential to contribute to hormone equilibrium(Hanf and Gonder 2005, Chajes and Romieu 2014), potentially diminishing the likelihood of hormone receptor-positive breast cancers. Animal models play an important role in drug discovery and mechanistic studies.

Breast cancer research relies on diverse animal models, each tailored for specific investigations. Spontaneous models involve no treatment (Rao, Piegorsch, et al. 1987). Induced models use chemical agents like DMBA or MNU (Chan, Lu et al. 2007), physical factors such as radiation (Russo & Russo, 1996), or biological methods like lentivirus infection (Fisher, Orsulic et al. 1999, Bu, Xin et al. 2009). Transplantation models include homotransplantation, where breast cancer cells are transplanted into the same strain (Paschall and Liu 2016), and heterograft models, involving the transplantation of human cells or patient tumour tissues into immunodeficient animals (Burdall, Hanby, et al. 2003). Genetic engineering mouse models encompass transgenic models with oncogene activation (Rashid and Takabe 2015) and knockout models with tumour suppressor gene inactivation (Hutchinson and Muller 2000). This variety of models enables comprehensive studies of breast cancer, facilitating a deeper understanding of its complexities and potential therapeutic strategies.

Spontaneous breast tumour models in mice and rats are pivotal for advancing our understanding of breast cancer biology, offering insights into the natural progression, latency, frequency, and pathology of these tumours. Notable models include the C3H mouse strain with a 6–10 month latency, exhibiting high tumour frequencies in breeding females (95%) and virgin mice (88%) and presenting adenocarcinoma as the predominant pathology(Heston and Vlahakis 1971). The ‘A’ mice strain, despite unspecified pathology, demonstrates significant tumour frequencies in breeding females (80%–84%)(Strong 1936). The DBA/2 mouse strain presents varied tumour frequencies (female mice: 72%, virgin mice: 48%), offering a unique perspective on spontaneous tumour development(Szymanska, Lechowska-Piskorowska, et al. 2014). BALB/c mice show a 12-month latency, with lower tumour frequencies (female mice: 5%, virgin mice: 1%) and adenocarcinoma as the prevalent pathology(Heston and Vlahakis 1971, Machida, Sudo et al. 2019). In rat models, the

SHN strain exhibits high tumour frequencies in both breeding (97.2%) and virgin rats (88.3%), emphasizing adenocarcinoma as the consistent pathology(Nagasawa, Yanai, et al. 1976). The TA2 mouse model, with a short latency of 329.81±95.3 days, presents an intriguing 84.1% tumour frequency, warranting further exploration(Sun, Zhang, et al. 2008). Lastly, the Kunming mouse strain, with a 13.5-month latency, showcases a 25% frequency for invasive ductal carcinoma (IDC), contributing to a comprehensive understanding of breast cancer(Zheng, Zhou et al. 2014). In conclusion, these models provide a dynamic platform for studying breast cancer intricacies, and their variations offer nuanced perspectives, essential for unravelling complexities and developing effective therapeutic interventions.

In the field of research, the predominant method for studying mammary tumours involves using athymic mice or rat models induced through cell transplantation. However, an alternative approach utilizing spontaneous mammary tumour rodent models could offer certain advantages. Such models could offer insights into the transmission of mammary tumours through milk, mirroring observations seen in certain early-stage mice models(Johal, Ford, et al. 2011).

The hairless rat employed in this study is a result of a spontaneous mutation occurring in the outbred colony of the Sprague Dawley-NIN (SD/NIN) rat strain. This particular strain has been maintained at the Indian Council of Medical Research (ICMR)-NIN animal facility in Hyderabad, India, for over 50 years. The mutation arose as a spontaneous model within the SD/NIN rat colony and has been deliberately propagated through a selective breeding strategy and characterized across more than 50 generations. To establish foundational data for ongoing research, the traits of the hairless SD/NIN rats were scrutinized, encompassing their growth curve and serum parameters associated with liver, kidney, and metabolism(Motha, Patil, et al. 2023). The NIN animal facility, operating under controlled conditions, selectively bred and housed mutant hairless rats along with their parents.

We aim to meticulously examine and analyse the spontaneous growth of tumours over time, with a specific focus on identifying patterns and understanding the dynamics associated with morbidity and mortality in the context of tumour development. We delve into intricate details along with skin histology, providing valuable insights for leveraging these animals in future research endeavours.

Materials and Methods

Study Design

In this research endeavour, a total of 24 SD/NIN rats were enlisted as subjects which were observed in their lifespan. This includes initial, physiology and serum parameters (at the age of 3 months) whereas these animals were maintained and observed for cage site observations until 18 months. These animals were observed for the occurrence of spontaneous tumours and their growth. The serum markers were not included in this particular study but can be considered in designing further studies. The ethical clearance from the IAEC was secured (Approval no # P40F/IAEC/NIN/11/2012) before the commencement of the experiment and the dermal architecture analysis was included in the current study based on the suggestion from the Scientific Advisory Committee of the institution (Project ID. 14-NC/03). Both male and female animals were equally represented by two distinct groups: the homozygous (hairless) rat group and the heterozygous group. The phenotype of this homozygous mutant (SD/NINhr-/hr-) animal is easily identified by the presence of hairless skin whereas heterozygous (SD/NINhr+/hr-) and homozygous wild (SD/NINhr+/hr+) will have good hair coat as it was a recessive gene-related character. Detailed genetic characterization is planned for further research.

Housing

Throughout their lifespan, these animals were closely observed for any phenotypical alterations. Rats were housed in open-style

polypropylene cages, with a controlled room environment such as a temperature of 22°C ± 2°C, a relative humidity range of 45-55%, and a 12-hour light-dark cycle. These animals had ad libitum access to RO water and a 20% protein-pelleted standard diet throughout the study phase.

A total number of 10 animals exhibited spontaneous tumour growth (hairless - 3 M/5F, heterozygous – 0M/2F). These animals were isolated from the group and meticulously monitored for laboured breathing, feed and water intake, and any kind of physical trauma as self-mutilating skin injuries. The animals found positive with spontaneous tumours were kept in bigger-sized polypropylene cages (29" x 19.5" x 9") along with autoclaved paper shreds and ad libitum access to feed and water for supporting their social behaviour (**Figure 1D**). Although as per guidelines for humane care, tumours should not grow beyond 25mm in size, however since this study was not about drug testing on tumours but it was about the size and progression of the tumour. During this study utmost care was taken to observe if any animal was off-feed or showing any signs of pain like a hunch back. The well-being of the animals was considered paramount such as providing adequate feed pellets on the cage floor, in addition to feed in hopper. Animals were observed and maintained considering humane endpoints (like >10% decrease in weight in a week, anorexia, respiratory infection, or laboured breathing) guiding decisions rather than focusing solely on tumour weight. Subsequently, these animals were humanely euthanized using CO_2 asphyxiation. This facilitated the collection of tumour tissues, which were then subjected to comprehensive histopathological staining (H&E) and volumetric analysis. This approach allowed for a thorough characterization of both the tissue and tumour mass.

Furthermore, both the heterozygous and homozygous hairless animals underwent euthanasia to facilitate an evaluation of hair follicle structure and distribution patterns. Additionally, the immuno-characterization aspect of the study involved the use of the BD FACs Aria-II system. This system enabled the quantification of B and T cell populations, contributing to a more holistic understanding of the immune response.

Histology

The skin and tumour tissues collected from both SD/NINhr$^{+/-}$ and SD/NINhr$^{+/+}$ rats underwent fixation in 4% buffered formalin for over 48 hours. Following fixation, the tissues underwent a series of alcohol changes and xylene treatment to facilitate paraffin embedding. Paraffin blocks were then used to obtain 4µm thick sections using a Leica Biosystems' rotary microtome, which were subsequently employed for subsequent staining. The sections placed on slides were subjected to staining using Haematoxylin and Eosin stains and were subsequently observed under various magnification objectives.

FACS analysis for Lymphocyte quantification

The FACS methodology for quantifying CD4/CD8 cells involved the collection of blood samples through the retroorbital plexus puncture method and subsequent storage in K-EDTA tubes. BD Biosciences Kit and BD Aria-II were utilized for the CD4 and CD8 cell quantification. Initially, a 100 µl aliquot of whole blood was transferred into a 5ml BD FACS tube. Subsequently, antibodies conjugated with fluorescein isothiocyanate (FITC) linked to mouse anti-rat CD3 (Cat. No:559975, clone G4.18), phycoerythrin (PD) anti-rat CD4 (Cat. No:554838, clone OX-38), and perCP mouse anti-rat CD8a (Cat. No:559976, clone OX-8) were added and thoroughly mixed in the tube. This mixture was incubated in the dark at room temperature for 15 minutes. Following incubation, 2mL of 1x FACS lysis buffer (BD Biosciences, Cat. No. 349202) was added and incubated again for 20 minutes in the dark at room temperature. After the incubation period, the solution was centrifuged for 5 minutes at 1200 RPM, and the supernatant was removed to eliminate cellular debris. Subsequently, 2 mL of sheath solution (BD Biosciences) was added and used to wash

the pellet in the centrifuge tube, with the washing step repeated twice. The washed cells were analysed using a flow cytometer (BD FACS Aria-II). To ensure accurate analysis, samples were gated based on forward vs 90° light scatter, allowing for the exclusion of granulocytes and monocytes from peripheral blood cells.

Results

Two methodologies were utilized to bolster colony expansion. Initially, a homozygous male was paired with a heterozygous female, yielding a 48% frequency of strains manifesting the hairless phenotype. The subsequent approach involved mating a heterozygous male with a homozygous female, resulting in 44% of progeny exhibiting the hairless trait. Scaling up the aforementioned experiment with a larger sample size would yield more precise outcomes and mitigate any bias stemming from pre-weaning mortality. The latter technique, employing a homozygous female, was executed to investigate whether it presents analogous issues to certain strains of hairless mice (athymic), characterized by non-functional mammary glands. However, no such resemblance was observed. The phenotype of homozygous exhibits naked skin (without hair) from birth (**Figure 1A-1C**), whereas wild and heterozygous phenotypes will have hairy coats similar to SD/NIN rats.

Since the mortality of SD mutant rats was observed during the age of 18 months, we euthanized rest of the animals at the age of 18 months. Euthanasia was performed using CO_2 asphyxiation, and the animals were subsequently examined for both macroscopic and histological indications.

Necropsy Findings – Skin and Vital Organs

In-depth examinations were conducted on the hairless mutants derived from SD/NIN rats, designated as SD/NINhr rats. Intriguingly, these hairless rats exhibited no discernible morphological distinctions between homozygous and heterozygous counterparts at birth as seen in earlier publications (Motha, Patil et al. 2023). Notably, they retained normal vibrissae throughout their lifespans. Analysis of body weights revealed comparable measurements between hairless rats and normal heterozygous animals, with female hairless rats demonstrating slightly lower body weights compared to their male counterparts. Unexpectedly, the examination of hairless rats in this study unveiled remarkable revelations. Contrary to conventional expectations of hairless mutants lacking a thymus, necropsy results revealed the presence of a thymus gland in these rats. Equally significant, the remaining internal organs displayed typical appearances, appropriate colorations, and proportional sizes, devoid of noteworthy abnormalities. Skin histology evaluations provided insightful revelations. Regardless of the rats' age, homozygous animals showcased underdeveloped hair accompanied by prominent signs of atrophy, including atrophic dermal papillae, hair matrix, and internal and external root sheaths (**Figure 2A and Figure 2B**). Hair canals exhibited dilation and sporadic cystic formations, while the hair shaft demonstrated thinness and improper keratinization, particularly in the upper portion. Conversely, heterozygous rats exhibited typical skin attributes with regular hair follicles. In summary, the hairless rats examined in this study displayed a hairless phenotype that could be due to an autosomal recessive mutation, however detailed study is warranted. Despite the anticipated absence of a thymus, necropsy surprisingly unveiled its presence. Moreover, the remaining internal organs exhibited healthy appearances, and comprehensive histopathological assessments unveiled no significant lesions. Furthermore, these rats demonstrated immunocompetence, as evidenced by functional thymus and antigen expression.

The provided figures offer insights into rats' developmental stages, spanning from newborns to 21 days old (**Figures 1A,1B,1C**), highlighting their growth and maturation process. Another figure presents the microscopic features of skin tissue (**Figures 2A and 2B**), using Haematoxylin and Eosin staining to visualize cellular structures and tissue attributes.

Necropsy Findings – Tumour Growth and Histology

Throughout their entire lifespans, continuous cage-side observations unveiled the appearance of tumours in both groups and genders when the animals reached 9 months of age. Notably, female hairless rats displayed a more pronounced occurrence of these tumours. These tumours were closely monitored until the animals reached humane endpoints indicated by signs such as breathing difficulties, loss of appetite, or a hunched back posture(Morton 2000). Importantly, the animals couldn't survive beyond 18 months, whereas the parent SD colony typically reaches 32 months of age. Shifting the focus to spontaneous mammary tumour development, a substantial discrepancy emerged between heterozygous and hairless rats. A total number of 10 animals exhibited spontaneous tumour growth (hairless - 3 M/5F, heterozygous – 0M/2F), however only four were analysed in detail (**Figure 4B**). These tumours are primarily localized in the ventral mammary gland region of female rats. Within this context, fibroadenoma, characterized by a combination of fibrous and glandular tissue, emerged as a benign growth within the breast. These tumours demonstrated mobility under the skin, were painless, and displayed a resilient, rubbery texture. Importantly, simple fibroadenomas did not elevate the risk of breast cancer. However, those containing macrocysts and calcifications exhibited an increased potential for progressing into breast carcinoma.

The size of tumours seen in the figures is huge because the rationale of the study was to observe the progression of the tumour until it reaches a humane endpoint concerning anorexia, hunched back, or inactive. A series of figures delve into fibroadenomas, employing various staining techniques (phyllodes, fibrous, calcification, cystic) to elucidate different aspects of tumour composition, growth, and potential complications (**Figures 3A – 3N**).

Collectively, these figures suggest a comprehensive study focused on rats with fibroadenomas, providing a thorough analysis of their development, structure, and composition. Using diverse staining methods and magnifications implies an extensive exploration of the tumours, potentially shedding light on their histological traits and clinical significance. While most fibroadenomas typically resolve on their own without requiring treatment, larger ones that exert pressure on surrounding breast tissues may necessitate surgical removal (Wilkinson, Anderson et al. 1989). Further details of the study could offer deeper insights.

CD4/CD8 Lymphocyte Quantification

Typically, hairless rat models are athymic, lacking a thymus and functionally mature T-cells, as well as the expression of T-cell associated antigens CD4 (helper T-cells) and CD8 (suppressor/cytotoxic T-cells). Intriguingly, our investigation into the fluorescence intensity of T-cell receptor complex-associated CD4 and CD8, about the immune study in the hairless mutant rat, revealed no significant cellular variations between both phenotypes. As a result, this hairless mutant rat displayed immunocompetence, being equipped with a functional thymus and antigen expression. There is no difference regarding an immunological picture of SD/NIN hairless rats (CD4%:65.4 ± 3.10, CD8%: 32.0 ± 2.89) concerning heterozygous SD/NIN rat (CD4%: 64.2 ± 2.41, CD8%: 32.5 ± 2.19). The fold change is shown in a graphical figure for further clarification (**Figure 4A and 4B**).

Figure 1A: New-born of SD/NINhr+/+: This figure likely displays new-born rats of the SD/NINhr+/+ genotype. It could be used to observe the appearance and characteristics of rats at birth.

Figure 1B: 14-days old Homozygous and Heterozygous SD/NIN hr rat: This figure illustrates the characteristics of 14-day-old SD/NINhr$^{+/-}$ and SD/NINhr$^{+/+}$ rats.

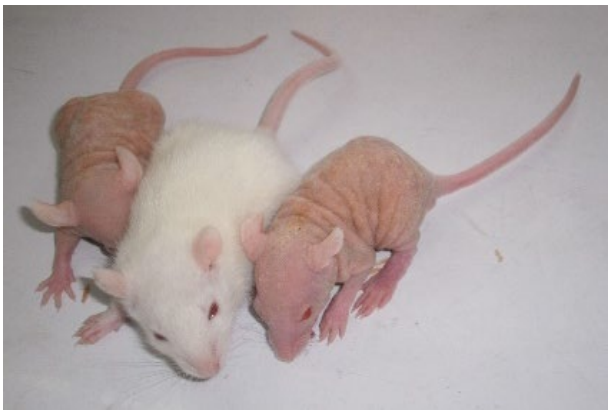


Figure 1C: 21-days old(weanlings) Homozygous and Heterozygous SD/NINhr rat: This depiction is to record the initial attributes for future comparison with subsequent developmental stages. It shows rats at 21 days old, weaned from their mother's milk. This stage marks significant growth and development.

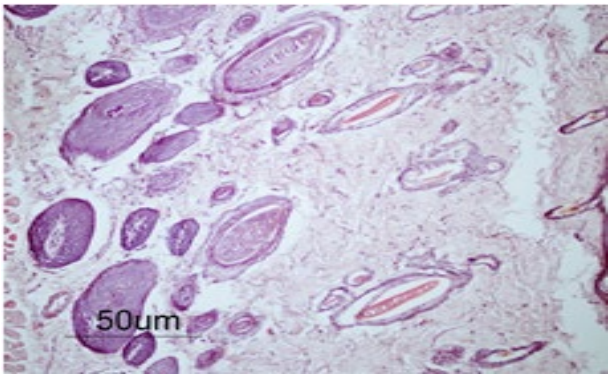


Figure 2A: Haematoxylin and Eosin staining of SD/NINhr$^{+/-}$ Skin.

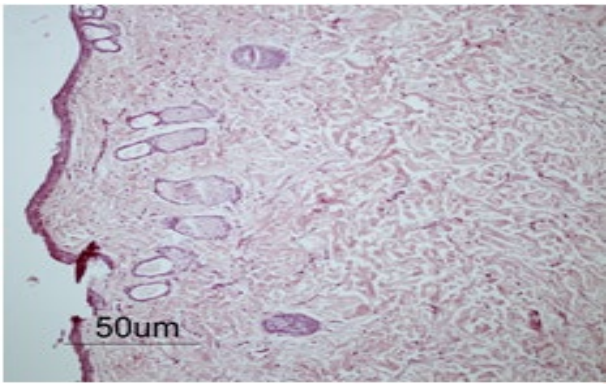


Figure 2B: Haematoxylin and Eosin staining of SD/NINhr$^{+/-}$ Skin.

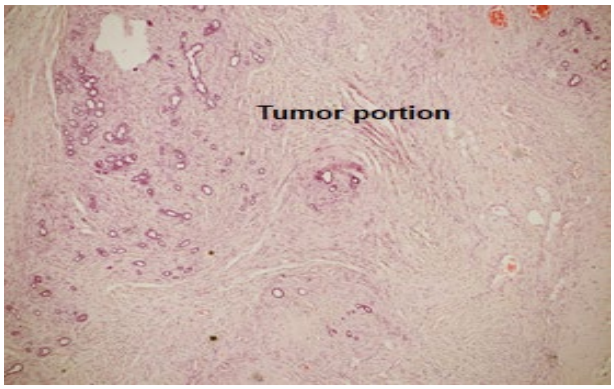


Figure 3A: Capsule in Fibroadenoma - H&E staining 10x: It showcase the tumour's boundary, providing insights into its encapsulation.

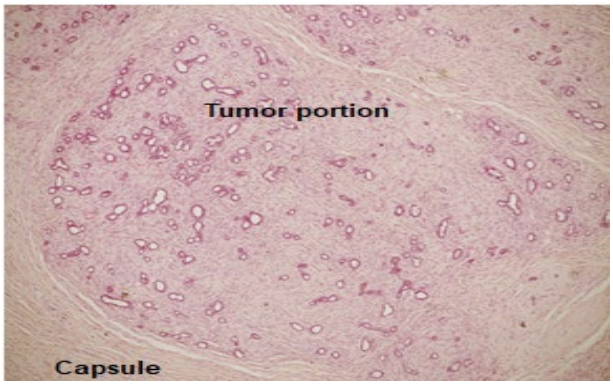


Figure 3B: Capsule in Fibroadenoma - H&E staining 40x: It showcase the tumour's boundary, providing insights into its encapsulation.

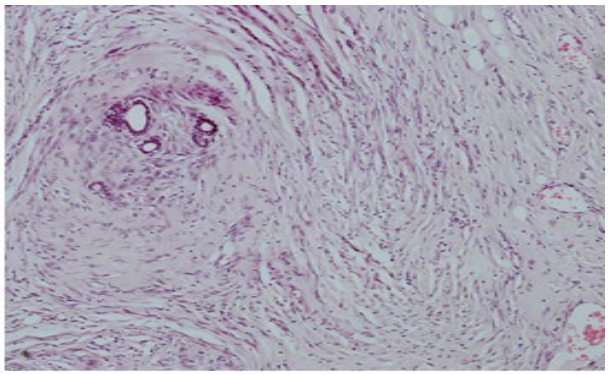


Figure 3C: Fibroadenoma - H&E staining 20x: These figures likely focus on different patterns within fibroadenomas, a type of breast tumour. The H&E staining helps visualize various characteristics.

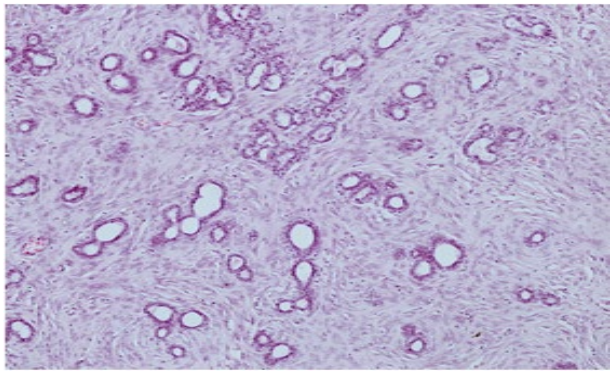


Figure 3D: Fibroadenoma - H&E staining 100x: These figures likely focus on different patterns within fibroadenomas, a type of breast tumour. The H&E staining helps visualize various characteristics.

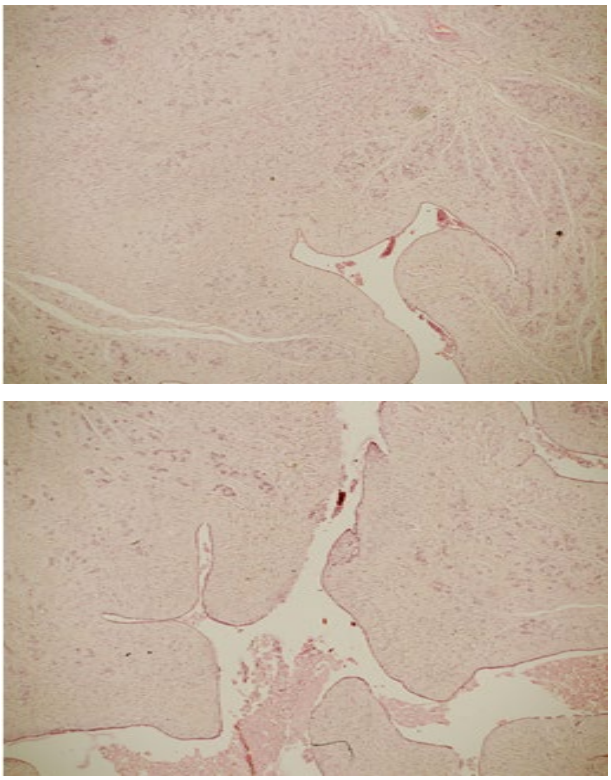


Figure 3E: Fibroadenoma H&E - phyllodes pattern 10x: Indicates a leaf-like growth pattern often seen in certain tumours.

Figure 3F
Fibroadenoma H&E - phyllodes pattern 40x: Indicates a leaf-like growth pattern often seen in certain tumours.

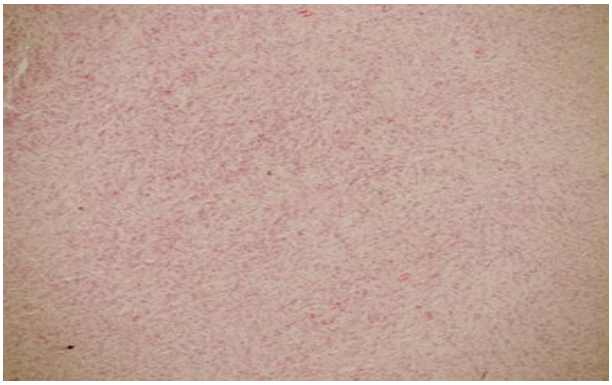


Figure3G: Fibroadenoma H&E - fibrous pattern 10x: Shows fibrous tissue within the tumour, potentially impacting its behaviour.

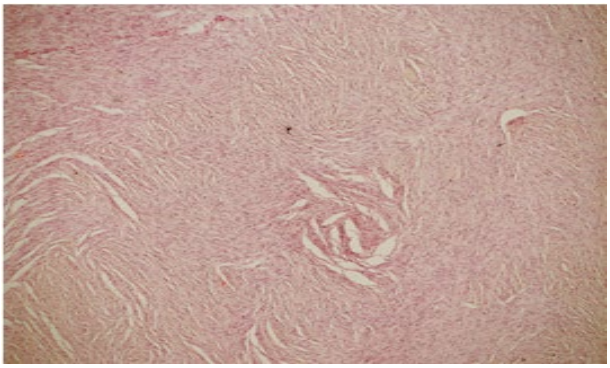


Figure3H:
Fibroadenoma H&E - fibrous pattern 40x: Shows fibrous tissue within the tumour, potentially impacting its behaviour.

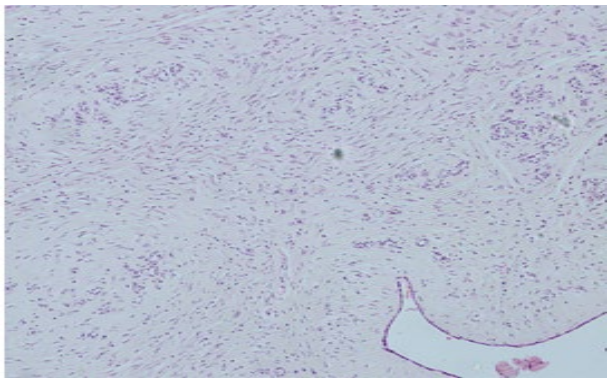


Figure 3I:
Fibroadenoma H&E - calcification 10x: Depicts the presence of calcified material, which could impact diagnosis and management.

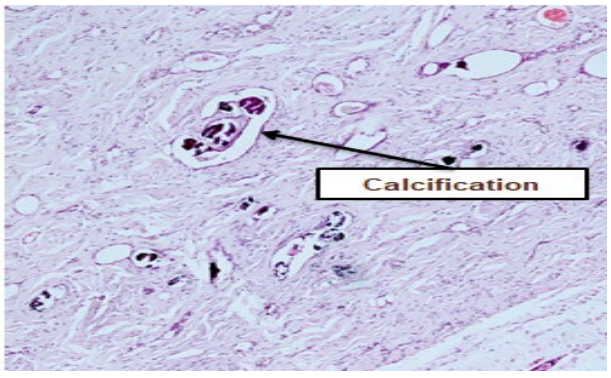


Figure 3J:
Fibroadenoma H&E - calcification 40x: Depicts the presence of calcified material, which could impact diagnosis and management.

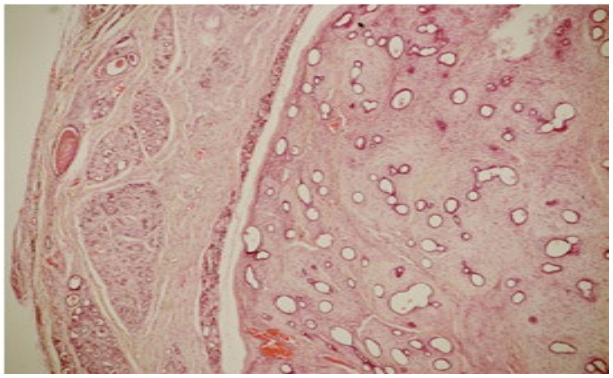


Figure3K:
Fibroadenoma H&E - cystic pattern 20x: Shows fluid-filled areas within the tumour, potentially indicating different tumour subtypes.

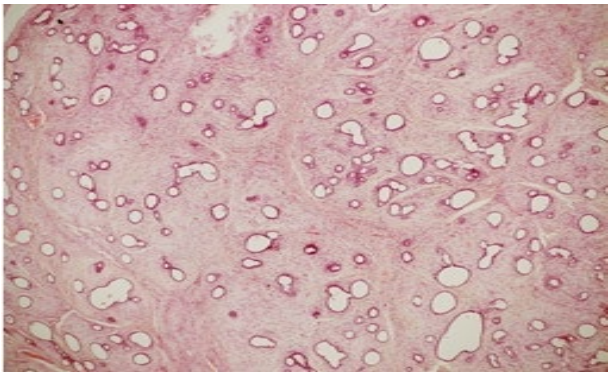


Figure 3l:
Fibroadenoma H&E - cystic pattern 40x: Shows fluid-filled areas within the tumour, potentially indicating different tumour subtypes.

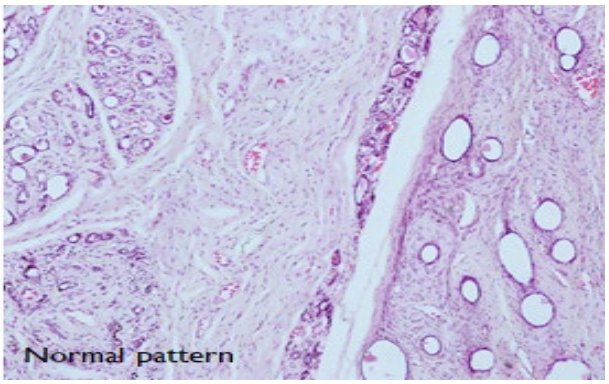


Figure3M: Fibroadenoma H&E - cystic pattern 100x: Shows fluid-filled areas within the tumour, potentially indicating different tumour subtypes.

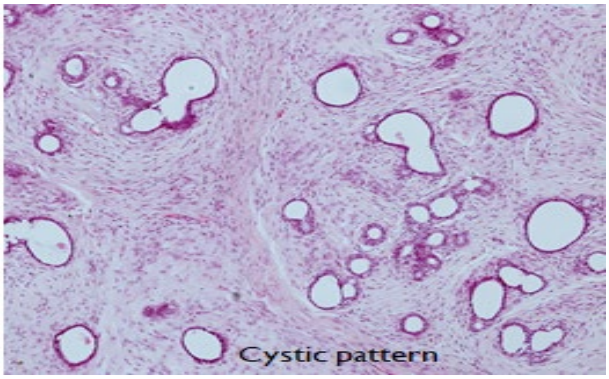
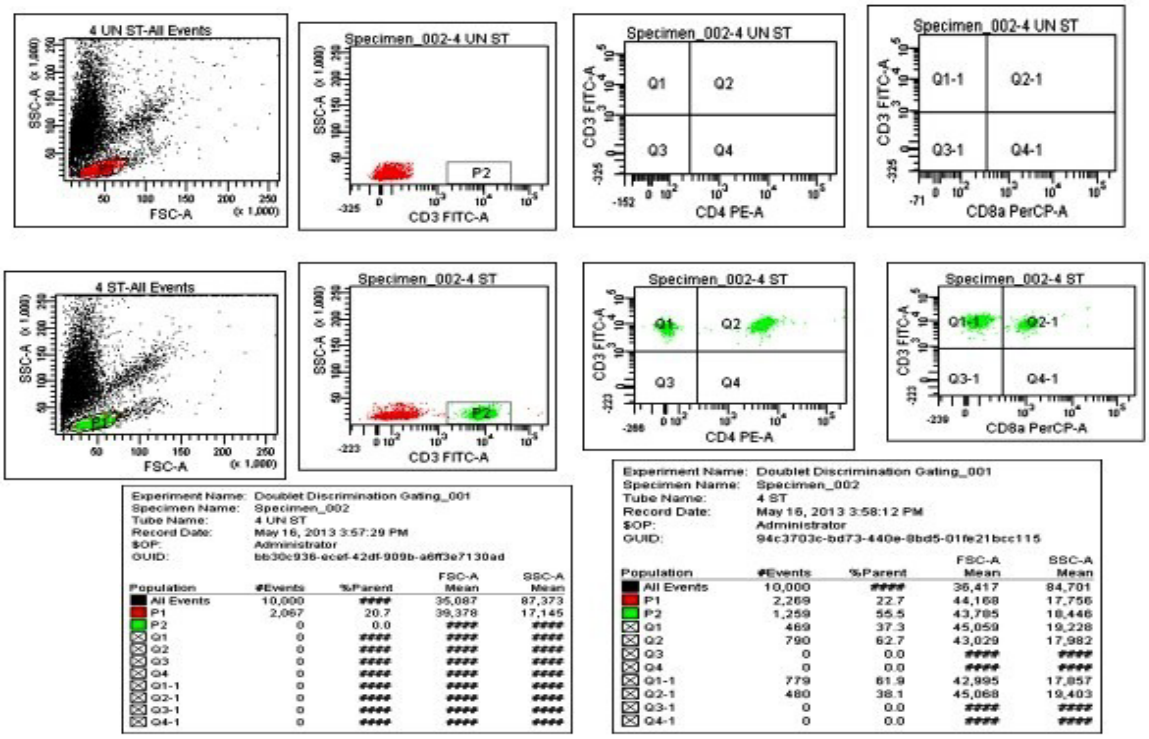


Figure3N:
Fibroadenoma H&E - cystic pattern 100x: Shows fluid-filled areas within the tumour, potentially indicating different tumour subtypes.



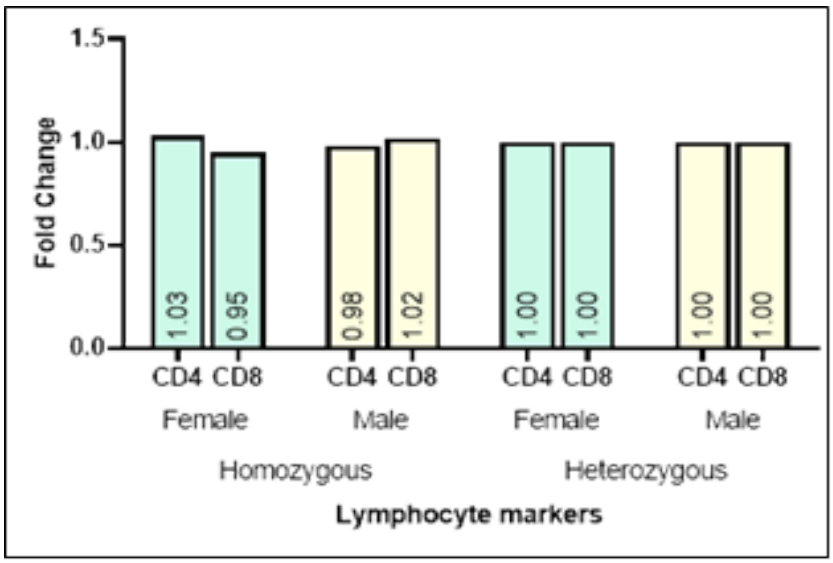


Fig 4A Fold Change and FACS analysis

Phenotype	Body Weight	Weight of the tumour (grams)	Tumour measurement		Tumour Volume (cubic mm)
			Length (mm)	Width (mm)	
Homozygous Female	409	76.9	74.72	44.65	74481.7
		50.2	50.09	46.89	55065.74
		13.1	33.13	32.9	17930.12
Homozygous Male	286	40.8	59.05	46.10	627465.3
Heterozygous Female	570	60.6	58.96	52.56	81440.
		172	102.55	79.07	3205746
Heterozygous Female	472	60	58.96	52.96	82684.37
		172	102.55	79.07	3205746.6

Figure 4B: Volumetry of Tumours

Discussion

This pervasive malignancy, characterized by the uncontrolled growth of cells within the breast tissue, transcends geographic boundaries and demographic distinctions, making it the most commonly diagnosed cancer among women globally(Coughlin and Ekwueme 2009). According to recent statistical data, the World Health Organization (WHO) estimates that in 2020 alone, approximately 2.3 million new cases of breast cancer were diagnosed, and remains the most common cancer worldwide (Arnold, Morgan, et al. 2022). This alarming prevalence underscores the urgent need for heightened awareness, early detection, and advanced treatment strategies in the ongoing battle against breast cancer. Employing appropriate rodent models, such as immunocompetent hairless rats as elaborated in this context, has the potential to enhance our comprehension of the interplay between transmission mechanisms and dietary factors in mammary tumours.

Interestingly, this study introduces a novel model of spontaneously developed mammary tumours. This model is particularly well-suited for cancer studies due to its immunocompetent nature, which closely

mimics real-life scenarios. Building upon our prior publication on the hairless rat (Motha, Patil, et al. 2023), where we examined its phenotype and physiological characteristics, this article serves as a continuation.

The data from an earlier study(Motha, Patil et al. 2023), conducted on these animals below 3 months of age revealed important information regarding Feed Intake and Body Weight, TOBEC, and Serum Biochemistry. Correlating this data with the current study, which includes animals above 3 months of age, provides a more comprehensive understanding of the subject. In terms of Feed Intake and Body Weight, Tukey's multiple comparisons test highlighted significant differences among experimental groups at various time points. Notably, differences in food intake were observed between homozygous female rats and heterozygous female rats, as well as between homozygous female rats and heterozygous male rats. Similar trends were evident at subsequent time points. Moreover, substantial variations in body weight were evident between different groups, emphasizing the influence of genotype and gender on food intake and body weight throughout the experimental timeline.

In terms of TOBEC measurements in female rats, homozygous rats exhibited negative percent changes in Body Weight and Fat Content, indicating reductions compared to the reference state. Conversely, heterozygous rats have maintained stability across all measured parameters. In male rats, there were noteworthy variations in TOBEC measurements between homozygous and heterozygous individuals. These changes encompassed parameters such as body weight, fat content, total body water content, fat-free mass, total body sodium, and total body potassium (explained in detail in earlier publication) (Motha, Patil et al. 2023). These changes shed light on nuanced distinctions in these parameters between the two genotypes.

As described in our earlier publication(Motha, Patil, et al. 2023), the analysis of Serum Biochemistry data in females unveiled intriguing variations. Notable reductions in glucose levels were observed in the homozygous group compared to the heterozygous group. Conversely, significant differences were not evident in bilirubin, total protein, and total glyceride levels. Urea levels showed a substantial increase in the homozygous group, potentially indicating differences in kidney functionality or nitrogen metabolism pathways. Albumin levels displayed a surge in the homozygous group, suggesting variations in liver performance or protein synthesis processes. Similarly, some enzyme levels and lipid metrics exhibited noticeable differences between the groups. The study's Serum Biochemistry analysis in males revealed similar patterns of change in various parameters between homozygous and heterozygous male rats. This data is important to consider while using this model for further studies.

Within the context of biomedical research, various hairless rat strains have emerged through spontaneous mutations, each tailored to specific research domains. These strains serve as invaluable tools, contributing to advancements in diverse fields. Histopathological evaluations of the studied animals indicated healthy vital organs with no significant lesions. Food intake trends revealed complex interactions between gender, genotype, and age. Body weight differences persisted across different time intervals, reflecting the intricate interplay of genetic and gender factors. TOBEC data illuminated alterations in fat content in homozygous rats, while heterozygous rats exhibited stable measurements.

Figures presented in the study provided insights into the comprehensive exploration of fibroadenomas in rats, spanning from their development to tumour presence and fibroadenoma characteristics. Prior research(Inazu, Kasai, et al. 1984, Nanashima, Yamada et al. 2015) has also observed the absence of hair in these mutants. Detailed descriptions of hairless models' skin features were provided. The SD/NINhr model showcased trends in tumorigenesis and declined longevity. Thus, the hairless rat model

holds significance for skin carcinogenesis and physiological, and biochemical studies.

The study's findings underscore the importance of spontaneous mutations in rodents, yielding diverse models that contribute to biomedical research. These models, including the hairless rat, exhibit similarities to humans and are suitable for various experiments. The characterization of the SD/NIN hairless rat model, as presented in this study, establishes baseline data and provides a foundation for future research.

Building upon earlier work(Motha, Patil, et al. 2023), this manuscript extends its contribution by presenting skin histology data and reporting on spontaneously grown tumours. Comparative data encompassing FACs, TOBEC, and tumours incidence and measurements between hairless and heterozygous SD/NIN rats were meticulously provided. Contrary to other hairless models (Hougen 1991), our model has shown the presence of thymus, which is unique for immunocompetency studies. The measurements of tumour dimensions and volumes (**Figure 4B**) in different phenotypes (homozygous and heterozygous) and sexes (female and male) are crucial for understanding tumour development and may have implications for further research in cancer biology and treatment strategies. These measurements were taken using a vernier calliper and volume was measured using the water displacement method.

In the realm of biomedical research, numerous rat models have been developed and harnessed, capitalizing on their compromised immune systems to delve into a wide spectrum of disease facets, immunological retorts, and therapeutic interventions. Among these, notable strains of hairless or naked rats have taken centre stage, each contributing uniquely to scientific exploration. The rnu/rnu Hairless Rat, originating from the Rowett Research Institute in Scotland(Schuurman, Hougen et al. 1992), bears a distinctive absence of thymus and T cell insufficiency, rendering it a cornerstone in cancer, transplantation, and immunological inquiries. The Hsd: RH-Foxn1rnu Rat, marked by a genetic anomaly in the Foxn1 gene (Kremen, Bez, et al. 2019), epitomizes hairlessness and immune system perturbation, underpinning research in immunology, skin biology, and transplantation.

Significantly, the Copenhagen (COP) Hairless Rat study findings underscore that T-cells lack involvement in countering mammary tumorigenesis (Korkola, Wood et al. 1997). This correlates with our findings, wherein our hairless rat model exhibited ample T-cells, yet mammary tumours emerged in the SD/NIN hr Rat strain. Noteworthy is the emergence of the novel SD/NINhr rat model, characterized by distinct hairlessness and a fully developed thymus, presenting as a promising candidate. Its potential lies in simplified propagation and the advantages it offers for immunocompetent studies, spanning diverse dimensions encompassing morphology, genetics, biochemistry, and immunology. Positioned at the intersection of convenience and comprehensive investigation, this model stands primed to make substantial contributions to the ever-evolving landscape of biomedical research.

In summary, this study introduces a novel hairless SD/NINhr rat model with spontaneously developed mammary tumours. The homozygous (hairless) rats exhibited the unique phenotype of hairlessness, contrary to conventional expectations of hairless mutants. Unexpectedly, these hairless rats retained a functional thymus, demonstrating immunocompetence. The study documented the spontaneous development of fibroadenomas in the mammary glands of homozygous rats, particularly in females, which displayed a higher prevalence of tumour occurrence. Histopathological analyses of these tumours revealed their benign nature, primarily composed of fibrous and glandular tissues. The comprehensive analysis of various parameters, including serum biochemistry, immune cell quantification, and body composition, highlighted significant differences between homozygous and

heterozygous rats, as well as potential gender-specific influences.

Conclusion

This unique rat model holds promise for cancer research, particularly in the area of mammary tumours, due to its immunocompetent nature that closely mirrors human scenarios. However, there is a limitation with this model that in humans Fibroadenoma is benign, and the majority time it requires no treatment. The model's spontaneously occurring tumours present an opportunity to delve into tumour development, progression, and potential therapeutic interventions. Overall, the hairless SD/NINhr rat model represents a valuable addition to biomedical research, offering insights into various aspects of physiology, immunology, and cancer biology.

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Conflict of Interest The authors declare no conflict of interest.

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