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Dissecting Physiological Regulation of Tumor Growth in *Drosophila*
(Merungkai Pengaturan Fisiologi Pertumbuhan Tumor dalam Drosophila (Lalat Buah))

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ABSTRACT

Drosophila melanogaster, also known as the fruit fly, serves as a powerful genetics model for dissecting tumor growth regulation. Traditionally, tumor development was thought to be driven solely by genetic mutations; however, epidemiological studies have shown a strong correlation with abnormal physiological conditions, such as diabetes. Numerous studies using *Drosophila melanogaster* have revealed important factors that influence tumor growth, such as insulin and inflammatory factors. Here, we propose a new conceptual design for a genetic tool to dissect the relationship between hormones and tumor growth. This system enables tumor induction with tissue-specific and temporal control, allowing us to analyze the underlying molecular mechanisms linking systemic physiology to local tumor growth.

Keywords: Tumor growth, Hormone, Genetic tool, *Drosophila melanogaster*

ABSTRAK

Drosophila melanogaster, yang juga dikenali sebagai lalat buah, berfungsi sebagai model genetik yang berkesan untuk merungkai kawal atur pertumbuhan tumor. Secara tradisinya, perkembangan tumor dianggap didorong semata-mata oleh mutasi genetik; walau bagaimanapun, kajian epidemiologi telah menunjukkan korelasi yang kukuh dengan keadaan fisiologi tidak normal, seperti kencing manis (diabetes). Banyak kajian yang menggunakan Drosophila melanogaster telah mendedahkan faktor-faktor penting yang mempengaruhi pertumbuhan tumor, seperti insulin dan faktor keradangan. Di sini, kami mencadangkan satu reka bentuk konseptual baharu bagi alatan genetik untuk merungkai hubungan antara hormon dan pertumbuhan tumor. Sistem ini membolehkan aruhan tumor dengan kawalan khusus tisu serta kawalan masa (temporal), sekali gus membolehkan kami menganalisis mekanisme molekul asas yang menghubungkan fisiologi sistemik dengan pertumbuhan tumor tempatan.

Kata Kunci: Pertumbuhan tumor, Hormon, Alat genetik, Drosophila melanogaster

METHODOLOGY

Fly Strains and Generation of Clones *Drosophila melanogaster* strains were raised in vials containing fly food. Normal diet is based on wheat flour composed of 35g of wheat flour, 10g of wheat germ, 50g of dried yeast, 52g of sugar, 7.2g of agar, 3g of sodium carbonate, 6mL of propionic acid, and 1g of methyl paraben in 1 L of MilliQ water. High sugar diet is composed of 20g of yeast extract, 20g of peptone, 80g of dried yeast, 342g of sugar, 7.5g of agar, 0.5g of MgSO4, 0.5g of CaCl2, 6mL of propionic acid, and 1g of methyl paraben in 1 L of MilliQ water. All ingredient was mixed in MilliQ water and autoclaved with stirring. Flies were maintained at 25 ºC, 12/12 light control. For the generation of cell clones of interest, 10-15 virgin females of healthy tester strains were crossed to 3-5 healthy males. Males and females at the wandering third instar larval stage were randomly collected for the assays.

RESULTS & DISCUSSION

The fruit fly, *Drosophila melanogaster*, is an invaluable model for understanding tumor growth due to its small body size, short life cycle, and versatile genetic tools (Fig. 1A). Despite the evolutionary distance between insects and mammals, many human cancer-related genes are conserved in the fly. For example, *scrib*, *Notch*, and *Ras* are essential for cell polarity, stem cell maintenance, and cell proliferation, respectively (1, 2, 3). While these genes are widely recognized as tumor drivers in humans, studies in *Drosophila melanogaster* have established their fundamental roles in animal development.

Drosophila offers distinct advantages for dissecting the functions of these genes. Unlike vertebrate model organisms, the fly genome exhibits less genetic redundancy, often possessing a single ortholog for a gene family. This simplicity facilitates a cleaner experimental design—such as single-gene knockdown—allowing for a clearer interpretation of gene function.

To leverage these advantages, we utilize a sophisticated genetic tool: the MARCM (Mosaic Analysis with a Repressible Cell Marker) system developed by Lee and Luo (4) (Fig. 1B). This system enables the generation of mosaic tissues composed of two distinct cell populations, allowing researchers to mimic the initial stages of tumorigenesis where mutated cells arise within a healthy tissue and to observe cell-cell interactions in vivo. In the MARCM system, tissue-specific induction of chromosomal recombination by Flp-FRT triggers the expression of the gene of interest via Gal4-UAS. Since this recombination event occurs stochastically, the target gene (e.g., a tumor driver) is expressed only in discrete clonal populations, while the surrounding cells remain in a normal state. Crucially, the Gal4-UAS system simultaneously drives the expression of Green Fluorescent Protein (GFP) alongside the gene of interest. This visible marker in manipulated tumor cells facilitates the identification of GFP-positive and negative clones within the tissue.

Figure 1C illustrates MARCM clones induced in *Drosophila* eye imaginal discs. When the system expresses only GFP (control), the resulting clones are considered wild-type, typically covering approximately 40% of the tissue. In contrast, GFP clones specifically knock down pre-tumorous gene appear significantly smaller than controls. This size reduction reflects the elimination of these "pre-tumorous" mutant cells by surrounding wild-type cells a surveillance mechanism known as cell competition, which functions as an intrinsic anti-tumor system (5). Conversely, when genetic manipulations mimicking aggressive tumorigenesis are introduced, the GFP clones expand rapidly and disrupt the original tissue architecture.

These complex genetic manipulations can be achieved through simple genetic crosses between a MARCM-ready strain and a UAS-transgene line. A single cross generates numerous offspring containing tumor clones in the eye disc, enabling highly efficient characterization of tumor growth. Collectively, these observations demonstrate that *Drosophila*, particularly when combined with the MARCM system, is an outstanding platform for the *in vivo* dissection of tumor development.

Cell competition functions as a tumor-suppressing mechanism, eliminating pre-tumorous cells through interactions with surrounding wild-type neighbors. This phenomenon was originally discovered in ribosomal protein mutant cells in *Drosophila* and has since been extended to include tumor-related genes such as *scrib*, *lgl*, *Myc*, *hpo*, *Ras*, and *Wnt* (6, 7, 8, 9, 10, 11, 12). Given that these genes are established tumor drivers in humans and that cell competition mechanisms are conserved in mammalian models, this process is potentially critical for controlling cancer risk in humans.

Figure 2A illustrates that *scrib* mutant cells, which lose apico-basal polarity, are recognized as pre-tumorous and efficiently eliminated by cell competition. Crucially, this elimination is observed only in healthy animals. In a model of hyperinsulinemia—characterized by high levels of circulating insulin—*scrib* cells evade elimination and instead develop into neoplastic tumors. Hyperinsulinemia is a hallmark of obesity and type 2 diabetes, conditions clinically associated

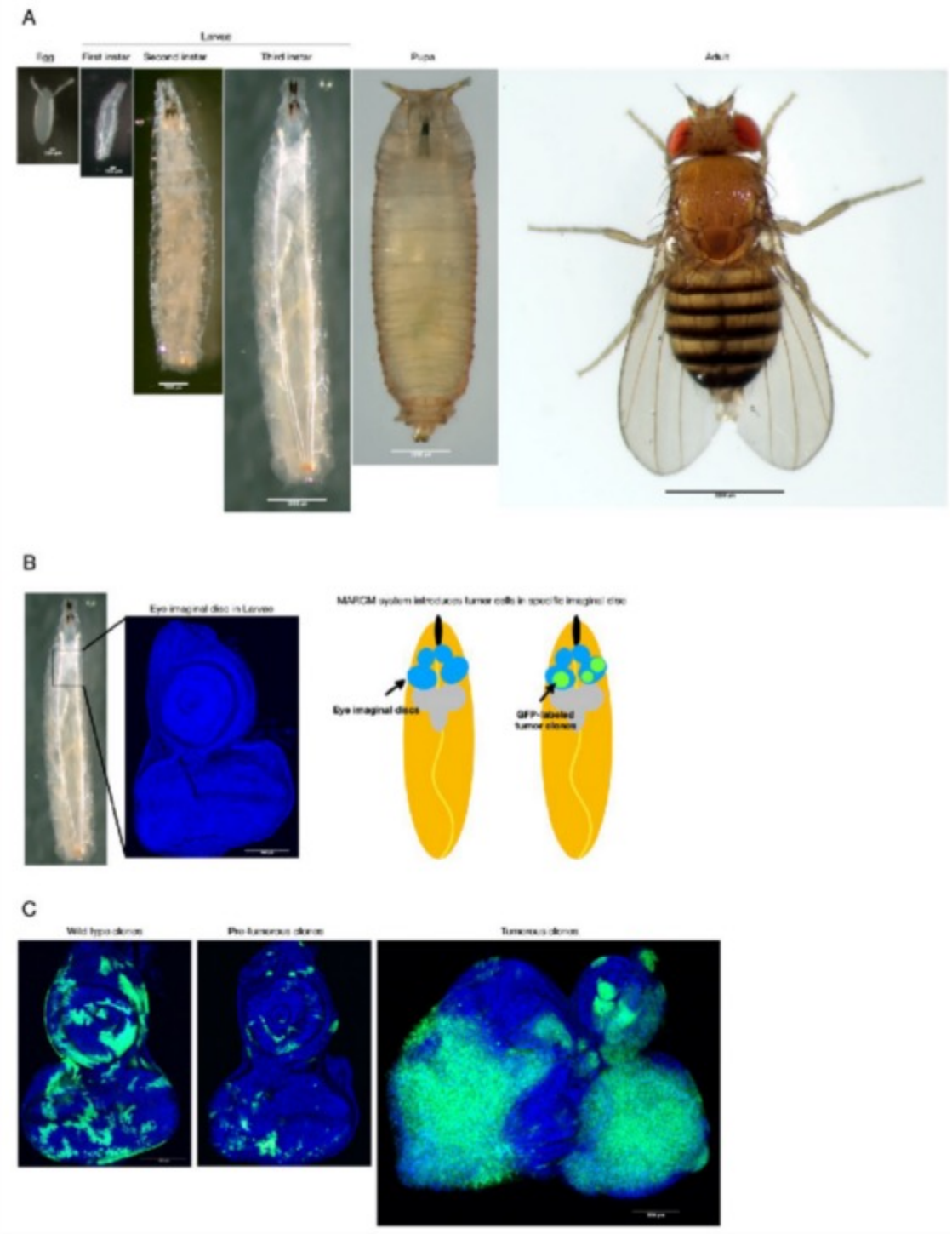

FIGURE 1. Drosophila melanogaster and tumor analysis by the MARCM system

A. The life cycle of *Drosophila melanogaster*. B. Eye disc in third instar larvae, which is often used for MARCM clone induction. C. MARCM clones induced in eye discs.

with high cancer risk (13). Thus, the compromise of tumor-suppressing cell competition in *Drosophila* recapitulates human epidemiology. Mechanistically, high levels of insulin directly stimulate *scrib* cells via the Insulin Receptor (InR) to evade cell competition (Fig. 2B)(14). Notably, this hyperinsulinemia state was induced by a genetic mutation in the insulin receptor substrate *chico*. This observation emphasizes the versatility of the MARCM system, which allows for the simultaneous analysis

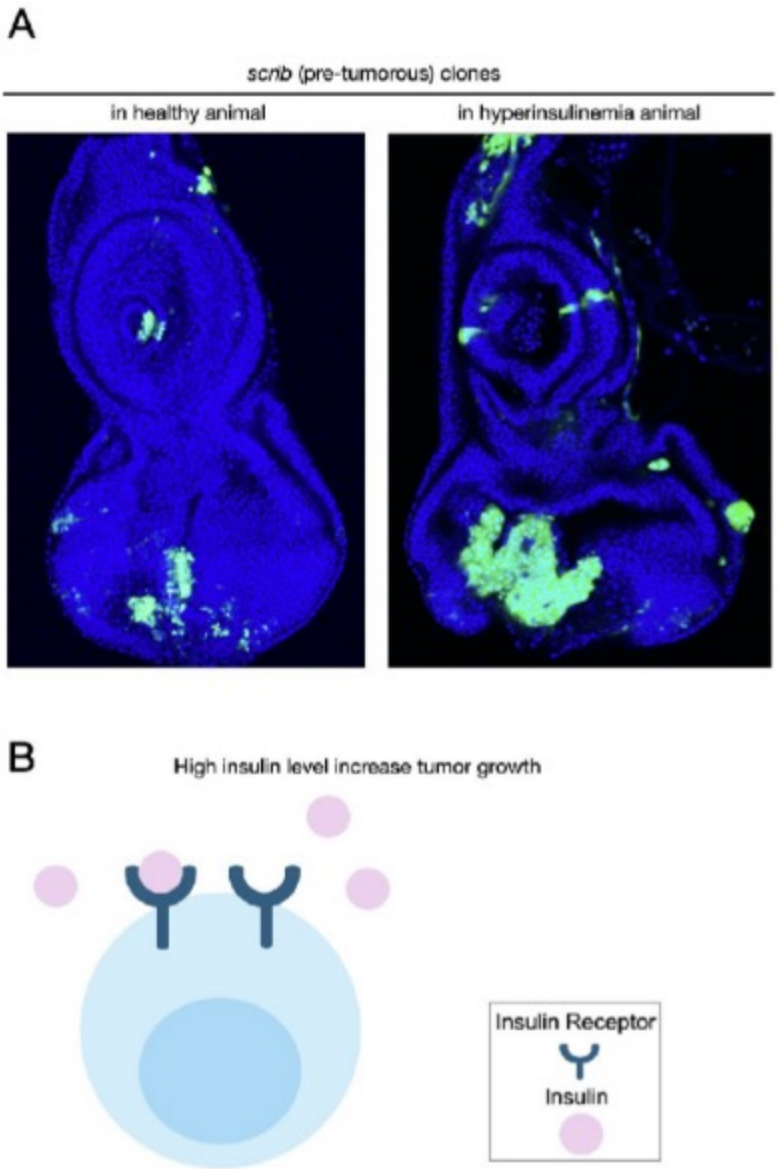

FIGURE 2. Tumor-suppressing cell competition is compromised by abnormal physiology, hyperinsulinemia

A. *scrib* mutant clones in control flies are eliminated, but overgrow into neoplastic tumors under hyperinsulinemia conditions. B. *scrib*-deficient cells exhibit high sensitivity to circulating insulin.

Beyond genetic manipulations, dietary factors profoundly influence animal physiology. Hirabayashi et al. revealed that high dietary sugar induces hyperinsulinemia, which in turn increases the aggressiveness of tumors (15). Figure 3A compares *csk*+*Ras*^{v12} tumor clones in larvae reared on a normal diet versus those on a high-sugar diet. Critically, tumor growth is significantly promoted under high-sugar conditions. Furthermore, animals fed a high-sugar diet exhibit secondary tumors (a metastasis-like phenotype), indicating an increased malignancy of the tumors.

While hyperinsulinemia is generally considered a promoter of tumor growth, we identified a striking exception in the aggressive cooperative tumor model combining *scrib* mutation with *Ras*^{V12} overexpression. Typically, *scrib*+*Ras*^{v12} tumors proliferate extensively and severely deform the eye disc morphology (Fig. 4A) (16). However, under high-sugar diet conditions, both total disc size and tumor clone size were significantly reduced compared to controls at the same time point. Time-course analysis revealed that while tumors on a normal diet exhibit exponential growth, those on a high-sugar diet appear to arrest growth 6 days after egg deposition, suggesting that the high-sugar environment

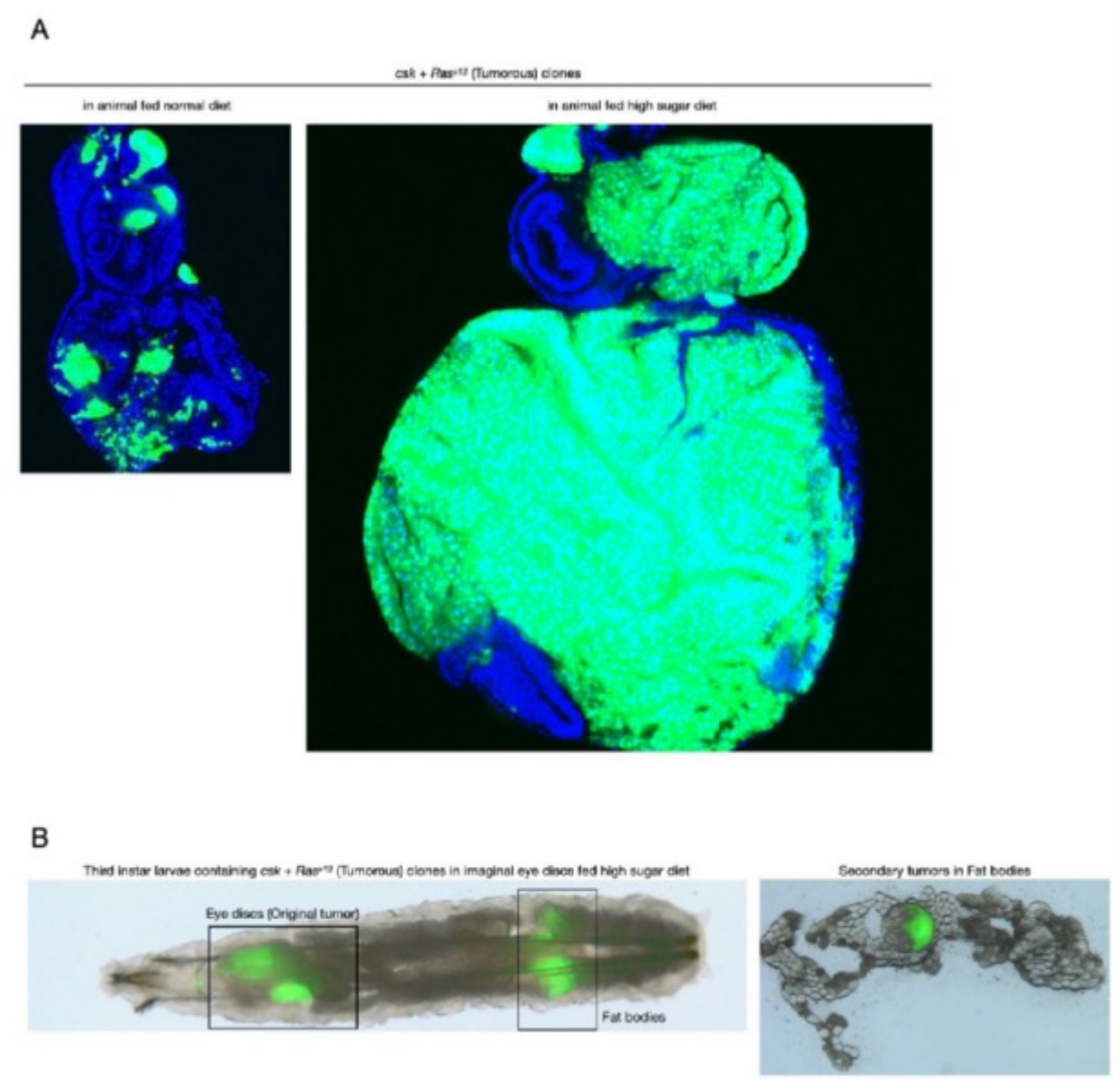

FIGURE 3. High sugar diet promotes *csk*+*Ras*^{v12} tumor aggressiveness

A. *csk*+*Ras*^{v12} clones are smaller on a normal diet but become aggressive tumor on a high sugar diet, B. secondary tumor formation on a high sugar diet.

suppresses this specific tumor type (Fig. 4B).

We also observed that tumor-bearing larvae reared on a high-sugar diet exhibited profound developmental arrest. On a normal diet, larvae began pupariation around day 5 AED, achieving a pupariation rate of approximately 20% despite the burden of the aggressive tumor (Fig. 4C). In contrast, *scrib*+*Ras*^{v12} larvae on a high-sugar diet failed to pupate even by day 13 (Fig. 4C). Since pupariation is controlled solely by the steroid hormone ecdysone, this result implies that ecdysone signaling is critical for tumor progression. We hypothesize that on a normal diet, systemic ecdysone levels reach a threshold sufficient to support both partial pupariation and tumor proliferation. However, the HSD condition likely dampens ecdysone production, failing to pupate and, consequently, an inability to support tumor growth (Fig. 4D). Indeed, it is established that tumor cells can actively incorporate circulating ecdysone via specific importers to fuel their metabolism (17).

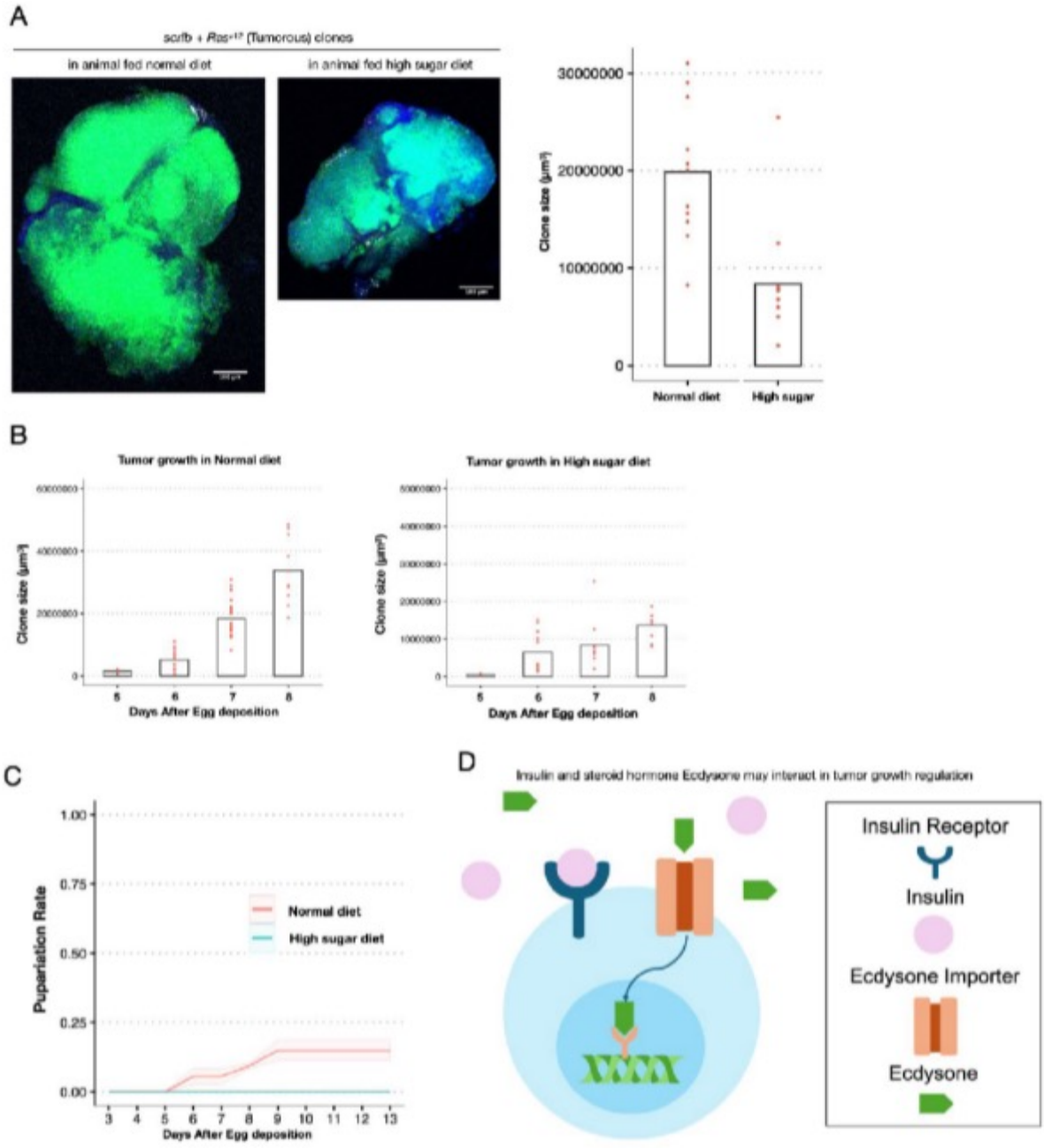

FIGURE 4. *scrib+Ras*^{v12} tumor did not grow in a high sugar diet but showed developmental failure

A. *scrib+Ras*^{v12} clones form an aggressive tumor on a normal diet but become smaller on a high sugar diet, B. tumor growth is exponential on a normal diet but arrested on a high sugar diet. C. partial pupariation on a normal diet but no pupariation on a high sugar diet. D. ecdysone may influence *scrib+Ras*^{v12} tumor growth.

While our results implicate ecdysone in tumor growth, circulating hormone levels fluctuate dynamically depending on the developmental stage and nutritional status. However, investigating these temporal dynamics is challenging with the standard MARCM system, as clone induction relies on tissue-specific promoters that are intrinsically coded expression timing (Fig. 5A). For example, the eyeless promoter, widely used for eye discs, initiates expression at an early embryonic stage. Due to this intrinsic constraint, the MARCM system precludes the precise temporal control of tumor initiation within a specific tissue.

To overcome this limitation, we are examining a novel mosaic analysis strategy that enables dual

spatiotemporal control of clone induction (Fig. 5B). This design integrates two independent recombinase systems: the conventional Flp-FRT system and a modified Flp-FRT (mFlp-mFRT) system (18). In this cascade, the first recombination event is triggered by a tissue-specific Flp (e.g., eyeless-Flp), similar to the standard MARCM system. This places a heat-shock promoter upstream of the modified Flp (mFlp) gene. Subsequently, the mFlp is activated by a temperature shift (e.g., 25ºC to 28ºC), triggering the recombination of mFRT sites flanking a STOP cassette. This second event initiates Gal4 expression, which finally drives the downstream expression of GFP and the gene of interest. This "double flip-out" method theoretically allows for the induction of UAS-driven clones with precise spatiotemporal control.

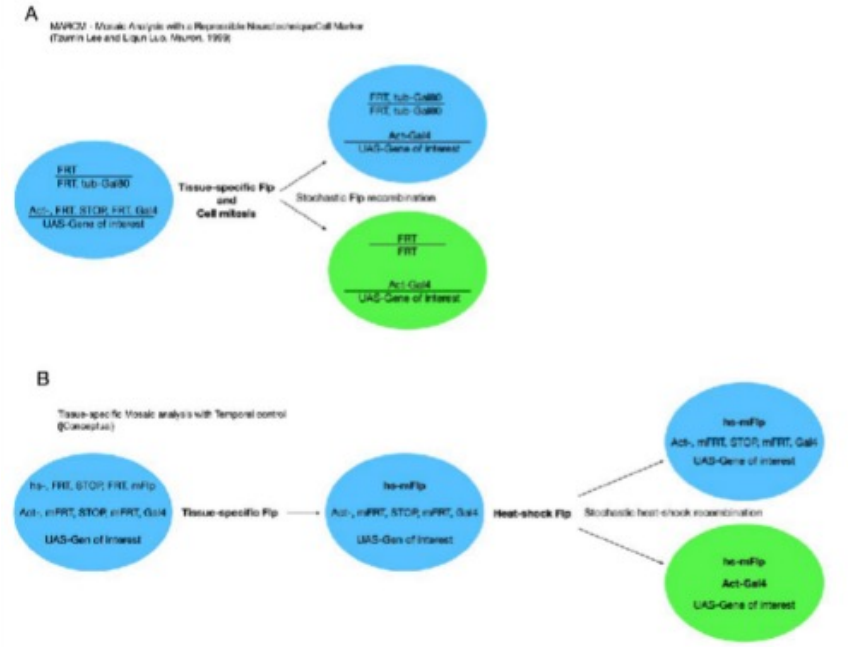

FIGURE 5. Schematic diagram of the MARCM system and the concept of tissue-specific Mosaic analysis with a temporal control system

A. MARCM system generates GFP-positive and negative clones by tissue-specific Flp recombination with mitotic recombination. B. Double Flip-out by tissue-specific Flp and heat-shock mFlp enables special and temporal control of clone induction.

CONCLUSION

Epidemiological studies have revealed that obesity and type2 diabetes are closely linked with high cancer risk. In humans, abnormal hormone levels and inflammation are associated with this cancer risk. Importantly, Drosophila melanogaster completely shares the same mechanism, namely hyperinsulinemia and JNK inflammation activation. This evidence indicates that studying cancer risk in Drosophila melanogaster strongly contributes to global health initiatives.

In this paper, we have highlighted the versatility of *Drosophila melanogaster* as a model organism for dissecting the complex mechanisms of tumor growth. Using the MARCM system, we demonstrated that tumor development is not solely dictated by cell-autonomous genetic drivers but is profoundly influenced by the host's systemic physiology. Specifically, our observations of the *scrib+Ras*^{v12} tumor model under high-sugar diet conditions revealed an unexpected suppression of tumor growth, likely linked to the failure of ecdysone

biosynthesis and developmental arrest.

These findings underscore the critical importance of temporal dynamics in hormone-tumor interactions. However, current genetic tools, which rely on developmentally fixed tissue-specific promoters, lack the temporal resolution required to precisely dissect these fluctuating hormonal effects. To overcome this limitation, we proposed a novel "Double Flip-out" strategy that integrates orthogonal recombinase systems to achieve precise spatiotemporal control of clonal induction. The development of this tool will allow researchers to trigger tumorigenesis at any developmental stage, enabling the direct interrogation of how specific hormonal environments, such as ecdysone peaks or insulin surges, impact tumor progression.

Furthermore, replacement of the transcription machinery with QF instead of Gal4 will allow researchers to utilize abundant fly genetic resources, mostly relying on the Gal4-UAS system to dissect the molecular mechanism. There are more than 37,000 UAS strains have been reported, which means the ways of genetic manupulation is exceed the number of genes in fly. The new “Double Flip-out” strategy with QF will make tumor clones in eye discs and enable the use of Gal4 to dissect inter-organ communication. This strong genetic tool will provide more evidence to connect fly studies and global health problems.

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