發明名稱

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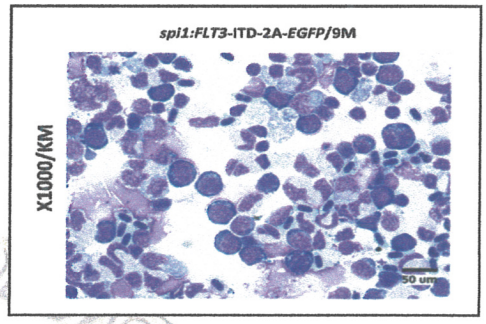

市場及需求:
急性骨髓性白血病（AML）是一種血液惡性腫瘤，且其致病機轉與治療預後具有相當大的差異性，最常見 AML 的突變乃是酪胺酸激酶接受器第三型，FMS-樣酪氨酸激酶 3（FLT3），其在造血過程中扮演著重要角色。近膜區中內部串聯重複（ITD）的發生將構成激活態 FLT3，約莫 20-25%AML 患者具有此一現象；並且在標準治療狀況下，病人反應較差。FLT3-ITD 的突變也被認為是一種骨髓性白血病的致癌突變；目前已經有一些 FLT3-ITD 抑制劑被用來治療 AML 病人。斑馬魚與人類具有非常相似的白血球組成，適合被用來當作疾病研究以及藥物開發之動物模式；目前還沒有任何 FLT3-ITD 的斑馬魚體內模式被開發來做為高通量藥物篩選平台。

技術摘要(含成果):
我們建立轉基因斑馬魚透過骨髓特異性啟動子控制表現人類 FLT3-ITD 或 NPM1-Mut 基因。具體來說，我們製作了兩個構築載體，分別稱為 spi1: FLT3-ITD and spi1:NPM1-Mut。Flt3-ITD 和 FLT3-ITD/NPM1-Mut 突變基因轉殖斑馬魚具有骨髓前驅細胞及芽細胞聚集與類似白血病的表癥。這些轉殖斑馬魚可應用於骨髓白血病和藥物探索的研究。野生株與 FLT3-ITD 斑馬魚腎髓與周邊血抹片的細胞學分析透露出，相較於野生株魚，6 與 9 個月大 spi1:FLT3-ITD 基因轉殖斑馬魚有更多的骨髓前驅細胞與過量的芽細胞聚集。其中，6 個月大 FLT3-ITD 基因轉殖斑馬魚腎臟結構已經出現輕微受壓而變小與歪曲以及骨髓細胞浸潤增加。進一步的骨髓抹片形態分析顯示在 9 個月大野生株魚具有正常的造血細胞之下，6 條 spi1:FLT3-ITD 斑馬魚中有 2 條具有不正常數量的骨髓前驅細胞與紅血球系列細胞減少。另外，在周邊血中也觀察到紅白母血球增加。我們建立的 FLT3-ITD 與 NPM1-Mut 雙基因(FLT3-ITD/NPM1-Mut)雙基因轉殖斑馬魚，在 6 個月大的時候即可看到前述現象。我們同時利用流式細胞儀及劉氏染色法來分析腎髓與周邊血液抹片，也可以發現 6 個月大的 FLT3-ITD/NPM1-Mut 雙基因轉殖斑馬魚，腎髓中芽細胞的大量增生同時伴隨著紅血球系細胞的減少，而不成熟的芽細胞可以逐步滲出腎髓，並且經由循環進入周邊血液。

優勢:

基於上述結果，我們聲稱 Flt3-ITD 與 FLT3-ITD/NPM1 斑馬魚模式可以被開發來做為高通量藥物的篩選平台。

競爭產品:

(1)沒有競爭的產品

專利現況:

(1)本疾病動物模型已申請美國臨時專利

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Title of Invention

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Experience:

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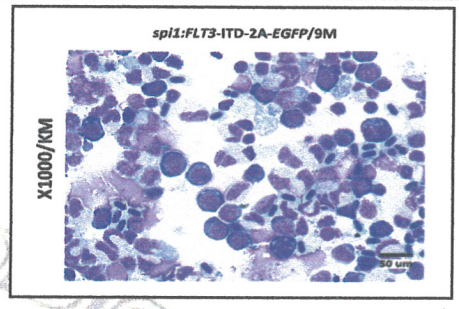

Market Needs:

Acute myeloid leukemia (AML) is a clonal hematologic malignancy that shows great variability with regard to pathogenesis and treatment outcomes. One of the most common mutations associated with AML involves the class III receptor tyrosine kinase, FMS-like tyrosine kinase 3 (FLT3), which plays an important role in hematopoiesis. Constitutively activated FLT3, occurring as internal tandem duplication (ITD) within the juxtamembrane domain, is observed in 20-25% of AML patients and refers to the poorest response to current standard treatment. *FLT3*-ITD mutant was reported to be a driver mutation in myeloid leukemogenesis. Zebrafish, sharing similar leukocyte compartment with human beings, recently emerged as a promising animal model for studying diseases and for drug discovery. In present, there are several FLT3-ITD inhibitors developed for AML patient. However, there is no in-vivo zebrafish model for high-throughput screening of FLT3-ITD inhibitors.

Our Technology:

We established a transgenic zebrafish that is able to express human *FLT3*-ITD or *NPM1*-Mut under the control of a myeloid-specific promoter (5.3 kb *spi-1*). Specifically, we generated two constructs, referred to as *spi1*: *FLT3*-ITD and *spi1*:*NPM1*, respectively. Cytological analysis of kidney marrow (KM) and peripheral blood (PB) smears prepared from wildtype and *FLT3*-ITD zebrafish were examined at 4, 6 and 9 months. In comparison with wild type fish, the KM from some *spi1*:*FLT3*-ITD 6- and 9-month old transgenic zebrafish had a greater number of myeloid progenitors and an excess of blast cells with focal aggregation. Hematoxylin and eosin (H&E) staining of the 6 month-old *FLT3*-ITD transgenic fish kidney showed mild effacement and distortion of kidney structure as well as increased infiltration of myeloid cells. Further morphological analysis of cytospin marrow showed that AB-wild type fish possessed the normal complement of hematological cells, whereas 2 out of 6 *spi1*:*FLT3*-ITD zebrafish had an abnormal number of myeloid progenitors

and decreased synthesis of erythroid cells at 9 month-old. Finally, leukoerythroblastosis was observed in PB.

We also generated *FLT3*-ITD/*NPM1*-Mut double transgenic mutants. In so doing, we found that *FLT3*-ITD and *NPM1*-Mut synergistically promoted myeloid blasts and the expansion of precursor cells in zebrafish as young as six months. Flow cytometric analysis and Liu's stains of the KM and PB smears from 6-month old *FLT3*-ITD/*NPM1*-Mut transgenic fish revealed myeloid hyperplasia with predominance of blast cells and depletion of erythroid series in KM, whereas immature blast cells were able to progressively infiltrate the KM and circulate into the PB. Taken above, we suggested that *FLT3*-ITD and *NPM1*-Mut synergistically promoted myeloid blasts and the expansion of precursor cells in zebrafish.

Strength:

Flt3-ITD and *FLT3*-ITD/*NPM1*-Mut transgenic mutants develop myelopoiesis with leukemic phenotype. These transgenic zebrafish strains can be useful in studies of myeloid leukemogenesis and drugs discovery.

Competing Products:

(1) There are no competing products.

Intellectual Properties:

(1) The disease model has been submitted to US provisional application

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