LYMPHOCYTE REGENERATION AFTER THYMOCYTE DAMAGE IN ZEBRAFISH

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Backgrounds

- Zebrafish's advantages:
  - developing in vitro
  - transparency
  - easy to image
  - easy genetic operation
  - large-scale genetic screening
  - relatively complete hematopoietic system
  - adaptive immune system

Significance

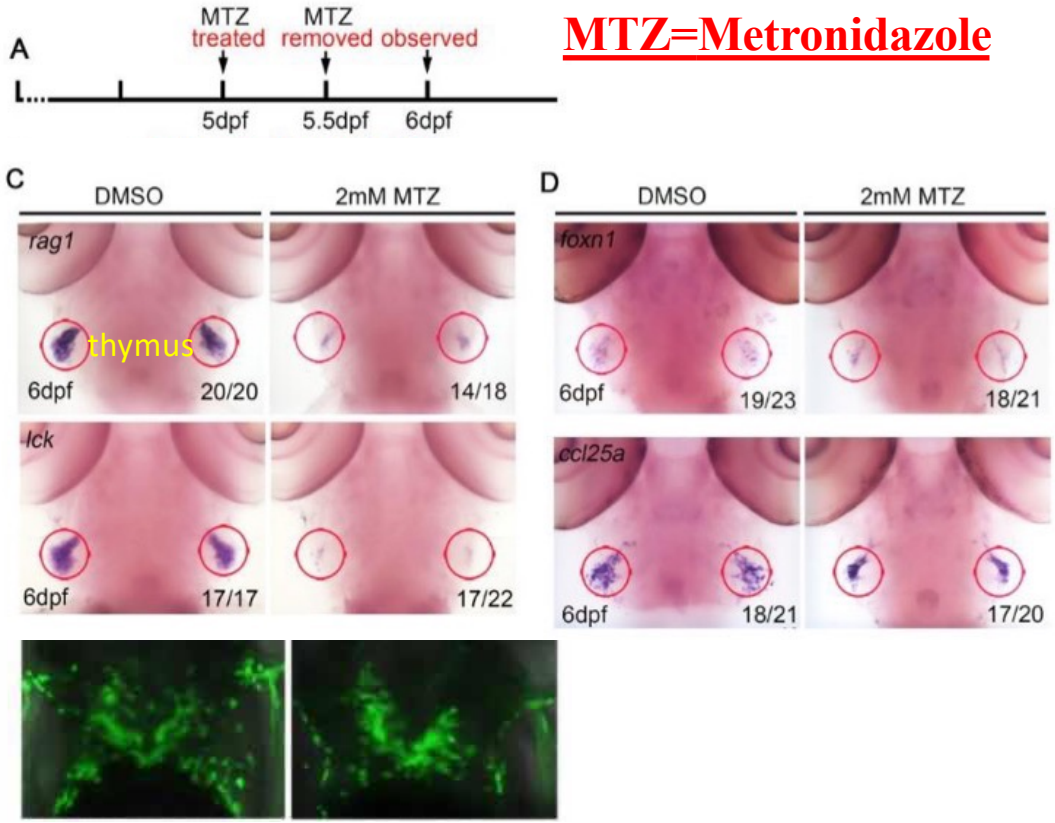
- As human aging, the thymus tissue shrinks, the number of thymic lymphocytes decreases, and the function of T cells decreases
- Radiation, chemotherapy, bacteria and DNA damage can also cause thymus atrophy and T lymphocyte defects, leading to impaired immune function

Construct a thymocyte damage model

Treatment: 2mM MTZ treatment on Tg (coro1a: Dendra2-NTR) transgenic zebrafish for 1-2 days

Results: (through WISH and confocal microscopy observations)

- lymphocytes were significantly reduced
- apoptosis occurred in thymus
- entire thymic epithelium was atrophied



Verifying lymphocytes regeneration

- *Tg(coro1a:Dendra2-NTR)×Tg(rag2-DsRed)*
- *Based on damage model*

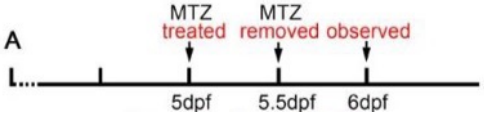
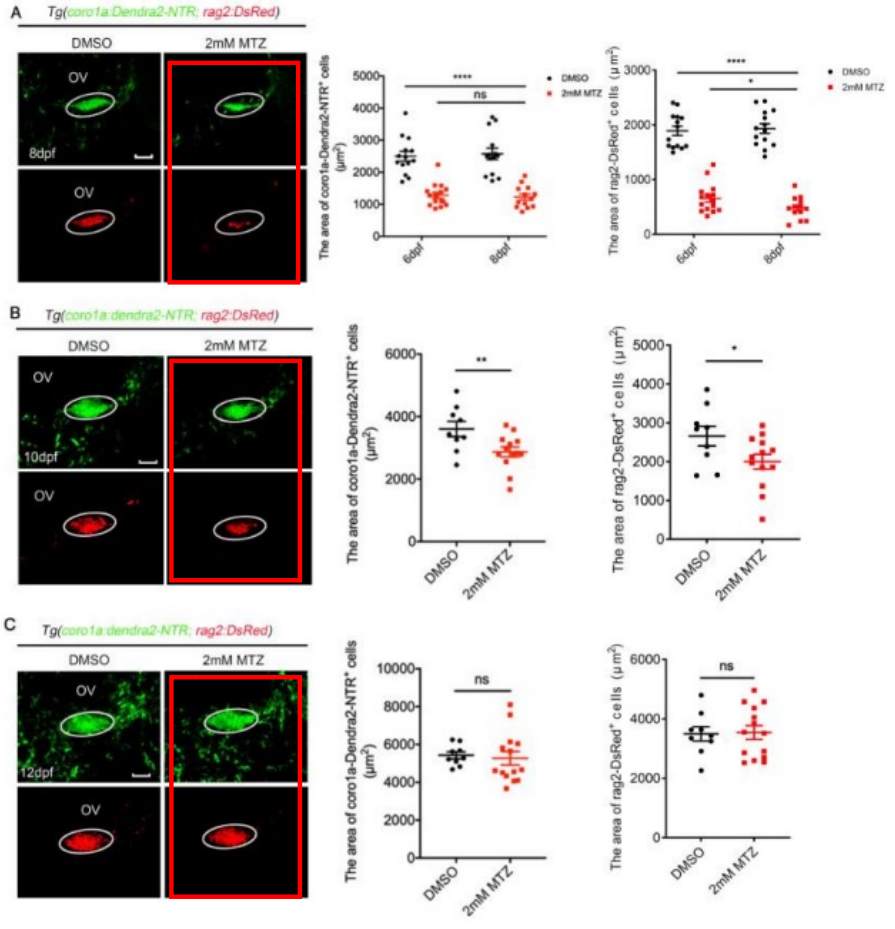


Fig A: 8dpf
T lymphocytes were still reduced

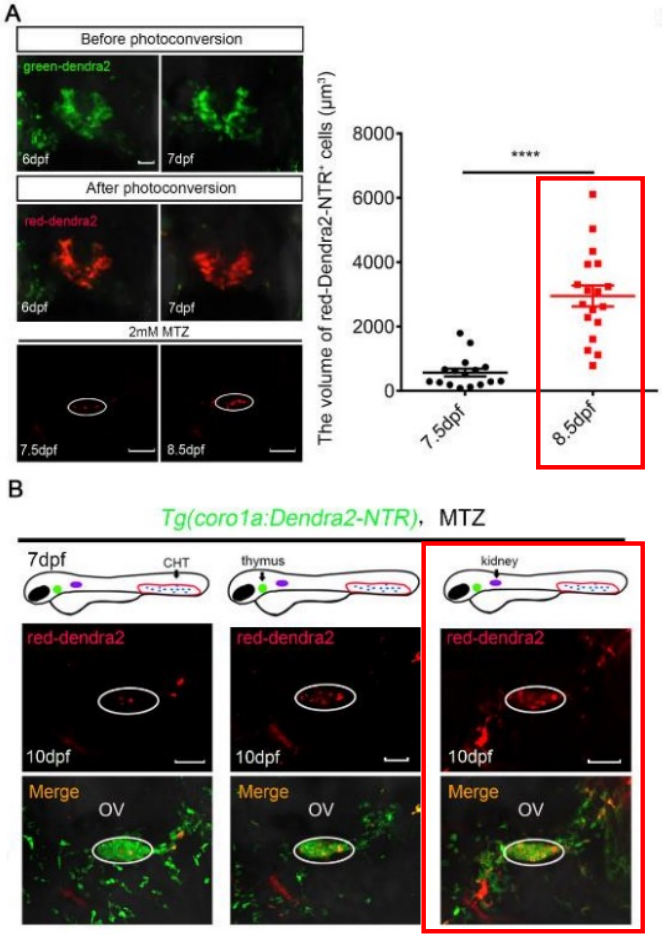
Fig B: 10dpf
Compared with the control group, there is still a big difference, and it has not returned to the normal level

Fig C: 12dpf
Returned to normal levels, both white blood cells and T cell area



Recovery process & the source

- Methods:
  - In vivo lineage tracing experiments under a confocal microscope
  - Under 405 nm UV excitation light, 6dpf and 7dpf kidney area hematopoietic cells (green-Dendra2+ cells) become red-Dendra2+ signal cells
- The results:
  - about 8dpf is the migration time point (dpf = day past fertilization)
  - most precursor cells migrate from the kidney to the thymus
  - little is produced in situ by thymus organs

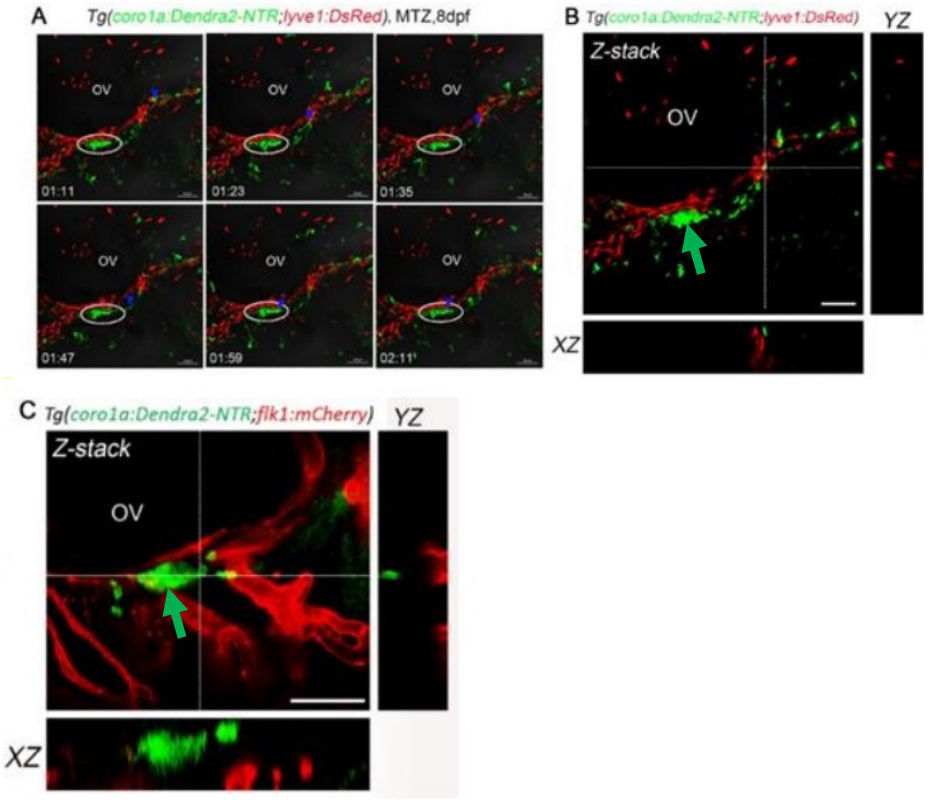


Regeneration pathway

- Known that:
  - T lymphocytes can be fully recovered on the 7th day after thymocyte injury, and mainly come from the lymphoid precursors migrated from kidney.
- Tg (coro1a: Dendra2-NTR) × Tg (lyve1: DsRed) [DsRed labels lymphatic vessels]
- Tg(coro1a:Dendra2-NTR) ×Tg(flk1:mCherry) [mCherry labels blood vessels]

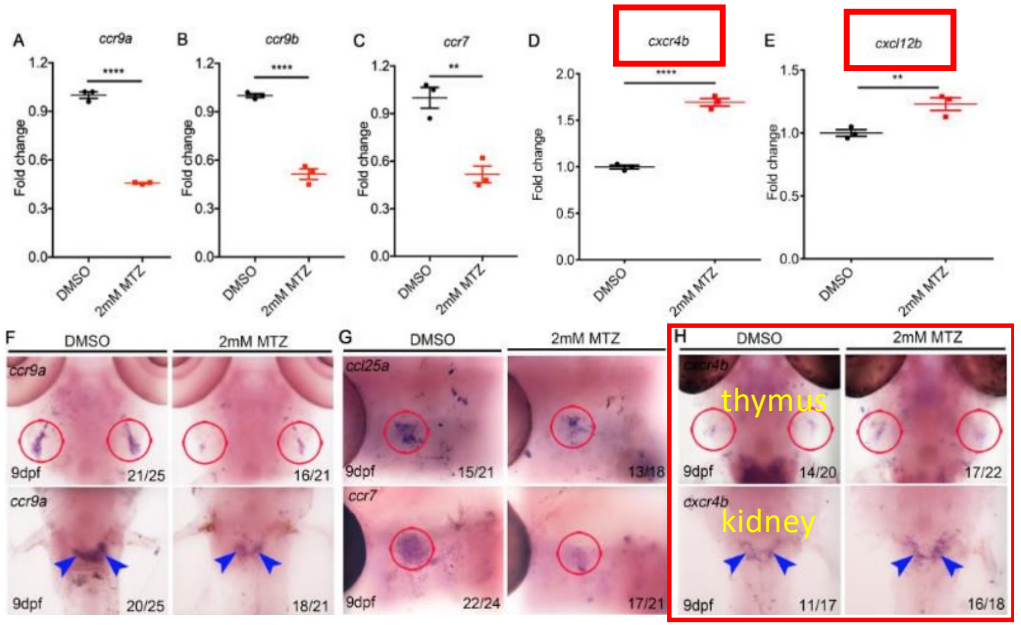
  → obtain double transgenic embryos with green &red fluorescent backgrounds.

  → Discover: the migration path of hematopoietic stem and precursor cells is through other ways besides blood vessels or lymphatic vessels.



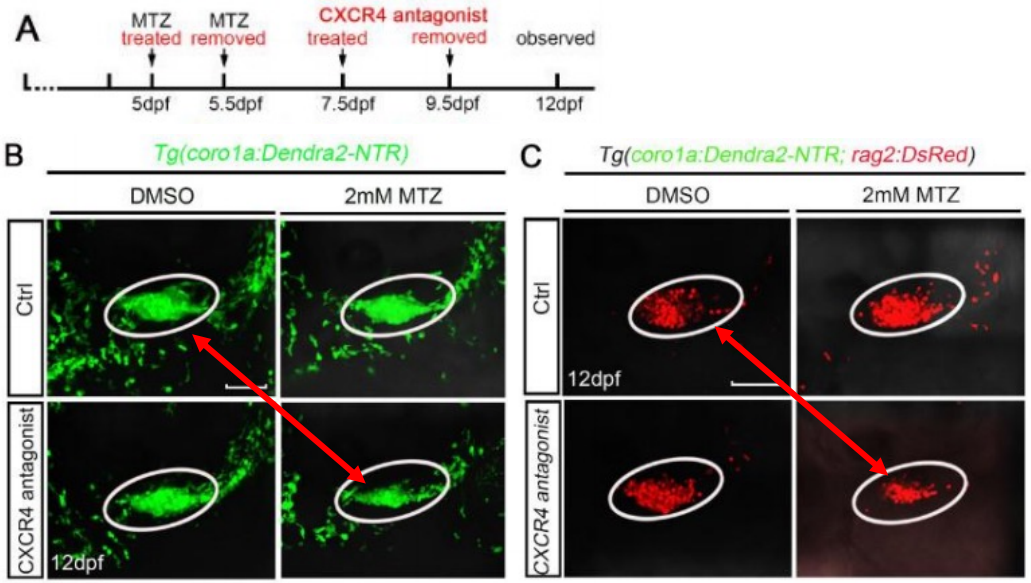
Up-regulated expressing signal pathway

- Methods: By qPCR detecting the expression of chemokine family genes and screening after MTZ in 9dpf
- Found: the chemokine receptor cxcr4b (expressed in lymphoid precursors) and cxcl12b (expressed in thymic epithelial cells) were significantly up-regulated during the regeneration of thymocytes.



Up-regulated expressing signal pathway

- Treatment of Cxcl12/Cxcr4 signaling pathway inhibitor.
- antagonist would hinder the migration of hematopoietic precursor cells from the kidney to the thymus, affecting the recovery of lymphocytes on the 7th day
- Cxcl12/Cxcr4 chemokines Signal pathways may play an important role in migration&recovery process



SUMMARY

- **Construct model: At 5dpf, 2mM MTZ treats Tg (coro1a: Dendra2-NTR) transgenic zebrafish for 1-2 days**
- **The regeneration of thymic lymphocytes mainly comes from the further differentiation of hematopoietic stem cells migrating from the kidney, and partly depends on the development of cells in situ**
- **After thymocyte injury, besides blood vessels or lymphatic vessels, the migration of hematopoietic precursor cells to the thymus is through other routes**
- **The Cxcl12/Cxcr4 chemokine signaling pathway may play an important role in the recovery of lymphocytes from the kidney to the thymus on the 7th day**

THANKS