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Title	Determining zebrafish dorsal organizer size by a negative feedback loop between canonical/non-canonical Wnts and Tlr4/NFκB
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論　文　内　容　の　要　旨
Synopsis of Thesis

氏　名 Name	ZOU JUQI
論文題名 Title	Determining zebrafish dorsal organizer size by a negative feedback loop between canonical/non-canonical Wnts and Tlr4/NFκB （古典的/非古典的Wntシグナルと Tlr4/NFκBシグナルの負のフィードバックループが背側オーガナイザーのサイズを決定する）

論文内容の要旨

〔目　的(Purpose)〕

In vertebrate embryos, the canonical Wnt ligand primes the formation of dorsal organizers that govern dorsal-ventral patterns by secreting BMP antagonists. In contrast, in *Drosophila* embryos, Toll-like receptor (Tlr)-mediated NFκB activation initiates dorsal-ventral patterning, wherein Wnt-mediated negative feedback regulation of Tlr/NFκB generates a BMP antagonist-secreting signalling centre to control the dorsal-ventral pattern. Although both Wnt and BMP antagonist are conserved among species, the involvement of Tlr/NFκB and feedback regulation in vertebrate organizer formation remains unclear. In this study, we investigated the role of Tlr/NFκB and feedback regulation in vertebrate organizer formation using zebrafish.

〔方法ならびに成績(Methods/Results)〕

We firstly investigated the NFκB activation using a novel transgenic reporter zebrafish and found that the NFκB reporter was activated in dorsal organizer region. And then by combinational analysis of both anti-sense morpholino (MO)-mediated knockdown and CRISPR/Cas9-mediated knockout, we found that one NFκB family protein Rel, restricts dorsal organizer formation. Knockdown of *rel* by MO induced dorsalization, whereas *rel* knockout mutants had no significant embryonic defects. To explain this inconstancy, we tested if genetic compensation occurs in the *rel* knockout mutant. Interestingly, we found that another *rel* homologue, *rela*, was upregulated in *rel* knockout mutant, but not *rel* MO-injected embryos. Moreover, knockdown of *rela* by MO enhanced the expression of dorsal markers and induced dorsalized phenotypes in *rel* mutant embryos, but not in WT embryos. These results indicated that the upregulation of *rela* compensates for the genetic loss of *rel* in mutants. Notably, *rel* knockout mutant showed no obvious defects after *rel* MO injection, indicating that *rel* MO specifically inhibits the function of *rel*. Using this specific MO, we further demonstrated that Rel negatively regulates canonical Wnt signaling through activating the transcription of a Wnt antagonist *fizb*, thus restricting dorsal organizer formation.

Next, we showed that the Toll-like receptor Tlr4 mediates NFκB activation during organizer formation using a specific chemical inhibitor and a dominant-negative mutant. In addition, forced activation of canonical Wnt signalling by a constitutively active β-catenin mutant (β-cat CA) dramatically enhanced NFκB reporter activity and *fizb* expression, which was reversed by Tlr4 inhibition, indicating that canonical Wnt signaling activates NFκB through Tlr4. By further functional analysis, we found that canonical Wnt signaling activates Tlr4/NFκB through the non-canonical Wnt ligand, Wnt5b.

〔総　括(Conclusion)〕

In this study, we demonstrate that a negative feedback loop between canonical/non-canonical Wnts and Tlr4/NFκB determines the precise size of zebrafish dorsal organizer. In early zebrafish embryos, Wnt/β-catenin signalling stimulates the transcription of the non-canonical Wnt5b ligand, which activates the NFκB protein Rel through Tlr4 in the dorsal region. Rel stimulates the transcription of the Wnt antagonist *fizb*, thereby restricting the Wnt/β-catenin-active area and dorsal organizer size. Similar to *Drosophila*, zebrafish determine their embryonic DV axis through negative feedback regulation between Tlr/NFκB and Wnt. Interestingly, the roles of these factors appear to be switched between these two species. Tlr/NFκB acts as the initial cue of DV axis formation in *Drosophila* and as a feedback mediator in zebrafish, whereas Wnt functions as the initial cue in zebrafish and as a feedback mediator in *Drosophila*. Therefore, by combination of in vivo imaging, morpholino-mediated knockdown and CRISPR/Cas9-mediated knockout, we succeeded in uncovering the hidden function of Tlr/NFκB in vertebrate dorsal organizer formation.

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

TLR-NFκB経路は、自然免疫を作動させる細胞内情報伝達システムとしてよく知られているが、もともとは昆虫の胚の背と腹の違い（背腹軸）を生み出す因子として三十年以上前に発見された経路であった。昆虫での発見を契機に脊椎動物の背腹軸誘導におけるTLR-NFκB経路の機能解析が行われてきたが、遺伝子ノックアウト解析では期待する結果を得ることができず、その関連は証明されていなかった。申請者は、イメージング解析に適したモデル脊椎動物である小型魚類ゼブラフィッシュを用いてTLR-NFκB経路の活動を可視化する独自のアプローチを行い、その結果、初期胚においてTLR-NFκB経路がWnt/β-catenin経路と同じ領域で活性化することを初めて突き止めた。さらに、従来の遺伝子ノックアウト法では遺伝的補償により遺伝子機能を解析しにくいことに気づき、遺伝的補償を回避できる方法で機能解析を進めた結果、TLR-NFκB経路がWnt/β-catenin経路の活動を適切に抑制することで、シュペーマン-マンゴルドオーガナイザーのサイズ、ひいては背と腹の境界が適切に形成されることを突き止めた。このように、独自の実験系を用いて脊椎動物発生の新たなメカニズムを明らかにしており、博士（医学）の学位論文として十分価値あるものと認められる。