

pEF-BOS DDX3-HA (225–662) was made by using primers DDX3D2-3 (CTC GAG CCA CCA TGC AAA CAG GGT CTG GAA AAA C) and DDX3C R-Ba. To make pEF-BOS DDX3-HA (225–484) and pEF-BOS DDX3-HA (485–663), the primers DDX3D2 R-Ba (GGA TCC AAG GGC CTC TTC TCT ATC CCT C) and DDX3D3 F-Xh (CTC GAG CCA CCA TGC ACC AGT TCC GCT CAG GAA AAA G) were used, respectively. Reporter and internal control plasmids for reporter gene assay are previously described [26].

RNAi

Knockdown of DDX3 was carried out using siRNA, DDX3 siRNA-1: 5'-GAU UCG UAG AAU AGU CGA ACA-3', siRNA-2: 5'-GGA GUG AUU ACG AUG GCA UUG-3', siRNA-3: 5'-GCC UCA GAU UCG UAG AAU AGU-3' and control siRNA: 5'-GGG AAG AUC GGG UUA GAC UUC-3'. Twenty picomoles of each siRNA was transfected into HEK293 cells in 24-well plates with Lipofectamin 2000 according to manufacture's protocol. Knockdown of DDX3 was confirmed 48 h after siRNA transfection. Experiments were repeated twice for confirmation of the results.

Yeast two-hybrid assay

The yeast two-hybrid assay was performed as described previously [27]. The yeast AH109 strain (Clontech, Palo Alto, CA, USA) was transformed using bait (pGBKT7) and prey (pGADT7) plasmids. The transformants were streaked onto plates and incubated for 3–5 days. The IPS-1 CARD vector was constructed by inserting IPS-1 partial fragment encoding from 6 to 136 aa region into pGBKT7 multicloning site. Yeast two-hybrid screening was performed using human lung cDNA libraries. We obtained four independent clones, and one encoded DDX3 partial cDNA. SD-WLH is a yeast synthetic dextrose medium that lacks Trp, Leu and His aa. SD-WLHA lacks adenine in addition to Trp, Leu and His. SD-WL lacks Trp and Leu and thus non-selective plate.

Reporter assay

HEK293 cells (4×10^4 cells/well) cultured in 24-well plates were transfected with the expression vectors for IPS-1, DDX3 or empty vector together with the reporter plasmid (100 ng/well) and an internal control vector, phRL-TK (Promega) (2.5 ng/well) using FuGENE (Roche) as described previously [28]. The p-125 luc reporter containing the human IFN- β promoter region (–125 to +19) was provided by Dr. T. Taniguchi (University of Tokyo, Tokyo, Japan). The total amount of DNA (500 ng/well) was kept constant by adding empty vector. After 24 h, cells were lysed in lysis buffer (Promega), and the *Firefly* and *Renella* luciferase activities were determined

using a dual-luciferase reporter assay kit (Promega). The *Firefly* luciferase activity was normalized by *Renella* luciferase activity and is expressed as the fold stimulation relative to the activity in vector-transfected cells. Experiments were performed three times in duplicate (unless otherwise indicated in the legends).

PolyI:C stimulation

PolyI:C was purchased from GE Healthcare company, and solved in milliQ water. For polyI:C treatment, polyI:C (50 μ g/mL) was mixed with DEAE-dextran (0.5 mg/mL) (Sigma) in the culture medium, and the cell culture supernatant was replaced with the medium containing polyI:C and DEAE-dextran. Using DEAE-dextran, polyI:C is incorporated into the cytoplasm to activate RIG-I/MDA5.

Virus preparation and infection

VSV Indiana strain or poliovirus type 1 Mahoney strain were used for virus assay. Vero derived cell (Vero-SLAM) was used for propagation and plaque assay for VSV Indiana strain or poliovirus type 1 Mahoney strain. HEK293 cells were infected with viruses at MOI = 0.001 in a 24-well plate. The virus titers of culture media at indicated hours post infection in the figures were determined by plaque assay using Vero-SLAM cells. In some experiments that require rapid virus propagation, high MOI (0.1 ~ 1) was used for infection.

Immunoprecipitation

HEK293FT cells were transfected in a 6-well plate with plasmids encoding DDX3, IPS-1, RIG-I or MDA5 as indicated in the figures. Twenty-four hours after transfection, the total cell lysate was prepared by lysis buffer (20 mM Tris-HCl (pH 7.5) containing 125 mM NaCl, 1 mM EDTA, 10% glycerol, 1% NP-40, 30 mM NaF, 5 mM Na₃VO₄, 20 mM IAA and 2 mM PMSF), and the protein was immunoprecipitated with anti-HA polyclonal (Sigma) or anti-FLAG M2 mAb (Sigma). The precipitated samples were resolved on SDS-PAGE, blotted onto a nitrocellulose sheet and stained with anti-HA (HA1.1) monoclonal (Sigma), anti-HA polyclonal or anti-FLAG M2 mAb.

Confocal analysis

HeLa cells were plated onto cover glass in a 24-well plate. In the following day, cells were transfected with indicated plasmids using Fugene HD (Roch). The amount of DNA was kept constant by adding empty vector. After 24 h, cells were fixed with 3% of paraformaldehyde in PBS for 30 min, and then permeabilized with PBS containing 0.2% of Triton

X-100 for 15 min. For the polyI:C stimulation, 100 ng of polyI:C were transfected into HeLa cell in 24-well plates together with IPS-1 or DDX3 expressing vectors, and 24 h after the transfection, the cells were fixed and stained for confocal microscopic analysis. Permeabilized cells were blocked with PBS containing 1% BSA and were labeled with anti-Flag M2 mAb (Sigma), anti-HA polyclonal Ab (Sigma) or Mitotracker in 1% BSA/PBS for 1 h at room temperature. The cells were then washed with 1% BSA/PBS and treated for 30 min at room temperature with Alexa-conjugated Ab (Molecular Probes). Thereafter, micro-cover glass was mounted onto slide glass using PBS containing 2.3% DABCO and 50% of glycerol. The stained cells were visualized at $\times 60$ magnification under a FLUOVIEW (Olympus, Tokyo, Japan).

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Abbreviations: CARD: caspase recruitment domain · DEAD: Asp-Glu-Ala-Asp · DDX3: DEAD/H BOX 3 · IKK ϵ : I-kappa-B kinase ϵ · IRF-3: IFN

regulatory factor-3 · IP: immunoprecipitation · IPS-1: IFN- β promoter stimulator-1 · MDA5: melanoma differentiation-associated gene 5 · RIG-I: retinoic acid inducible gene-I · RLR: RIG-I-like receptor · TBK1: TANK-binding kinase 1 · VSV: vesicular stomatitis virus

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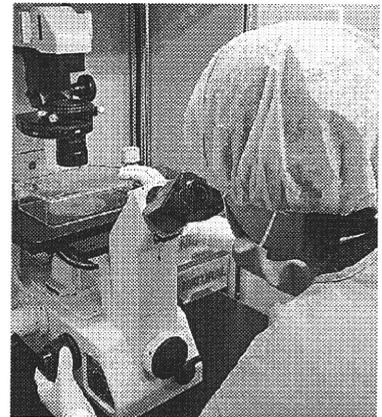
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医薬品が本業でないメーカーが独自技術を生かしワクチンの開発を進めている。東レはC型肝炎ワクチンを試作。森下仁丹は独自のカプセル技術で口から飲むタイプの腸チフスワクチンを開発する。新型インフルエンザの発生でワクチンに注目が集まるが、病気の流行状況などで需要が変動しやすい。参入組は安定需要が見込める分野に照準を定め、製薬会社と連携し実用化を目指す。

血液を介して感染し肝硬変になる恐れもあるC型肝炎。東レと国立感染症研究所などが試作したワクチンは、細胞を使う実験で日本人に多い「1b」型で66%、海外に多い「2a」型だと85%で感染を防げた。

技術ウオッチ



東レは独自技術を生かしC型肝炎ワクチンの実用化を目指す(国立感染症研究所)

非医薬、ワクチンに触手

人に備わる免疫機能を利用して感染症などを予防する。主に毒性を弱めたり、なくしたりした病原体をもとに作る。体の免疫系に病原体の特徴を伝え、病原体と闘う抗体を大量に作り出せるかが性能のポイント。注射タイプが多いが、途上国では投与しやすい経口型の潜在需要が大きい。

C型肝炎ワクチンを作るには原因ウイルスを大量培養し濃縮・精製する必要があるが、有効な手法がなかった。感染研がウイルスを培養できる細胞を見つけたのを端緒に、東レがウイルスに目印をつけ効率よく濃縮する手法を開発。「ウイルスを1万倍に増やし、さらに1万倍に濃縮できる」(東レの中村紀子主任研究員)方法で実用化の展望が開けた格好だ。同社にはC型肝炎治療用インターフェロン製剤を

量産するノウハウがあり、それをワクチン作りに活用する。患者は途上国を中心に毎年300万〜400万人増え、世界で推定1億7千万人。市場規模はB型肝炎ワクチンの600億円を

東レ、「肝炎用」開発にメド

日本では1980年代、集団予防接種時の事故で接種を控える人が増加。事業展望が読みづらくなり生産者もデンカ生研(東京・中央)など4社・団体にほぼ限られていた。だが海外製薬大手は予防医療の視点からワクチン事業の強化に動き、そこに独自技術を売り込む商機がある。

森下仁丹は神戸大学などと協力。「仁丹」で培ったカプセル技術を使い、飲む腸チフスワクチンの開発を進めている。

原因菌の遺伝子の一部をファイブス菌に組み込み、カプセルに封入。胃酸に弱いファイブス菌を腸まで運び、免疫系が抗体を作る。マウスの実験で治療に使え量の抗体ができることを

確認。コレラや赤痢などへの応用も視野に入れる。

日油は感染研などとインフルエンザウイルスに効くワクチンを開発中だ。ウイルス自体に働く従来ワクチンと違い、感染した細胞を攻撃する。実現にはウイルスのたんばく質を狙った場所に届ける技術が必要で、「優れた化学会社の協力が欠かせない」(感染研の内田哲也主任研究員)。

ただ承認を得るには大規模な臨床試験が欠かせず、日本ではワクチン全般で副作用を心配する人もいる。各社はまず海外の提携先を探す方針。東レの松田良夫主幹は「1〜2年以内に相手をを見つけ、臨床を見据えた動物実験に着手したい」と話す。

