特殊型炎症性腸疾患におけるアダリムマブとステロイドの前向き無作為化比較試験、
Castle Study： 国内多施設共同試験

◯渡辺憲治[1]、松本主之[2]、仲瀬裕志[3]、久松理一[4]、平井郁仁[5]、小林清典[6]、日比紀文[7]、渡辺　守[8]（大阪市立大大学院医学研究科消化器内科学[1]、岩手医科大学内科学講座消化器内科消化管分野[2]、京都大学消化器内科・内視鏡部[3]、慶應義塾大学医学部消化器内科[4]、福岡大学筑紫病院消化器内科[5]、北里大学東病院消化器内科[6]、北里大学北里研究所病院炎症性腸疾患先進治療センター[7]、東京医科歯科大学消化器病態学[8]）

B-(3)-3　外科治療の現状と工夫（14:35～15:30）

総括　杉田　昭　横浜市立市民病院外科

外科治療の現状、工夫、予後 -プロジェクト研究の現状と方針-

◯杉田　昭[1]、亀岡信悟[2]、二見喜太郎[3]、根津理一郎[4]、藤井久男[5]、楠　正人[6]、舟山裕士[7]、渡邉聡明[8]、福島浩平[9]、板橋道朗[2]、池内浩基[11]、佐々木巌[12]（横浜市立市民病院炎症性腸疾患センター[1]、東京女子医大第２外科[2]、福岡大学筑紫病院外科[3]、西宮市立中央病院外科[4]、奈良県立医科大学中央内視鏡超音波部[5]、三重大学消化管、小児外科学[6]、東北労災病院大腸肛門外科[7]、東京大学大腸肛門外科[8]、東北大学分子病態外科[9]、兵庫医科大学下部消化管外科[11]、みやぎ健診プラザ[12]）

クローン病術後療法に関する調査研究―インフリキシマブ併用療法・術後管理

◯福島浩平[1]、羽根田祥[2]、渡辺和宏[2]、長尾宗徳[2]、神山篤史[2]、鈴木秀幸[2]、舟山裕士[3]、杉田　昭[4]、二見喜太郎[5]、畠山勝義[6]、藤井久男[7]、吉岡和彦[9]、亀岡信悟[10]、渡邉聡明[11]、楠　正人[12]、池内浩基[13]、中村志郎[14]、鈴木康夫[15]、木内喜孝[16]、渡辺　守[17]、佐々木巌[18]（東北大学大学院消化管再建医工学・分子病態外科学分野[1]、東北大学大学院生体調節外科学分野[2]、東北労災病院大腸肛門外科[3]、横浜市民病院外科[4]、福岡大学筑紫病院外科[5]、新潟大学消化器・一般外科[6]、奈良県立医科大学中央内視鏡・超音波部[7]、兵庫医科大学外科[8]、関西医科大学付属香里病院外科[9]、東京女子医科大学第二外科[10]、東京大学腫瘍外科[11]、三重大学消化管・小児外科学[12]、兵庫医科大学IBDセンター外科[13]、兵庫医科大学IBDセンター内科科[14]、東邦大学医療センター佐倉病院内科[15]、東北大学保健管理センター[16]、東京医科歯科大学消化器病態学[17]、みやぎ健診プラザ[18]）

回腸嚢炎に関する調査研究―「寛解」の定義(案)

◯福島浩平[1]、羽根田祥[2]、渡辺和宏[2]、鈴木秀幸[2]、長尾宗紀[2]、神山篤史[2]、舟山裕士[3]、杉田　昭[4]、二見喜太郎[5]、藤井久男[7]、池内浩基[8]、小金井一隆[4]、飯合恒夫[6]、東大二郎[5]、吉岡和彦[9]、亀岡信悟[10]、板橋道朗[10]、渡邉聡明[11]、楠　正人[12]佐々木巌[13]（東北大学大学院消化管再建医工学・分子病態外科学分野[1]、東北大学大学院生体調節外科学分野[2]、東北労災病院大腸肛門外科[3]、横浜市民病院外科[4]、福岡大学筑紫病院外科[5]、白根健生病院[6]、奈良県立医科大学中央内視鏡・超音波部[7]、兵庫医科大学IBDセンター外科[8]、関西医科大学付属香里病院外科[9]、東京女子医科大学第二外科[10]、東京大学腫瘍外科[11]、三重大学消化管・小児外科学[12]、みやぎ健診プラザ[13]）

高齢者潰瘍性大腸炎に対する手術の検討 - 手術適応、手術時期、手術術式、予後のアンケート調査（中間報告） -

◯杉田　昭[1]、亀岡信悟[2]、二見喜太郎[3]、根津理一郎[4]、藤井久男[5]、楠　正人[6]、舟山裕士[7]、渡邉聡明[8]、福島浩平[9]、板橋道朗[10]、池内浩基[11]、佐々木巌[12]（横浜市立市民病院炎症性腸疾患センター[1]、東京女子医大第２外科[2]、福岡大学筑紫病院外科[3]、西宮市立中央病院外科[4]、奈良県立医科大学中央内視鏡超音波部[5]、三重大学消化管、小児外科学[6]、東北労災病院大腸肛門外科[7]、東京大学大腸肛門外科[8]、東北大学分子病態外科[9]、東京女子医大第２外科[10]、兵庫医科大学下部消化管外科[11]、みやぎ健診プラザ[12]）

クローン病肛門部病変の重症度分類について

◯二見喜太郎、東大二郎、石橋由紀子（福岡大学筑紫病院外科）

B-(4)　診療に伴う合併症/副作用および特殊型への対策プロジェクト

B-(4)-1　潰瘍性大腸炎合併サイトメガロウイルス腸炎および血栓症（15:30～16:15）

総括　鈴木康夫　東邦大学医療センター佐倉病院内科

潰瘍性大腸炎に対するインフリキシマブ効果予測と長期経過

○鈴木康夫、岩佐亮太、山田哲弘、竹内　健（東邦大学医療センター佐倉病院内科）

CMV感染合併潰瘍性大腸炎診断における大腸組織内CMV-DNA定量評価の意義 － 免疫組織染色法との比較検討 －

鈴木 康夫[1]、平山圭穂[1]、山田哲弘[1]、○仲瀬裕志[2]、石黒 陽[3]、大宮美香[4]、長沼　誠[5]、松岡克善[5]、長堀正和[6]、福知　工[7]、平井郁仁[8]（東邦大学医療センター佐倉病院内科[1]、京都大学消化器内科[2]、弘前病院消化器血液内科[3]、関西医科大学内科[4]、慶應義塾大学消化器内科[5]、東京医科歯科大学消化器病態学[6]、済生会中津病院消化器内科[7]、福岡大学筑紫病院消化器内科[8]）

潰瘍性大腸炎術後の消化管出血について（サイトメガロウィルス腸炎を含む）

福島浩平[1]、鈴木康夫[2]、羽根田祥[3]、渡辺和宏[3]、○神山篤史[3]、長尾宗紀[3]、舟山裕士[4]、杉田　昭[5]、二見喜太郎[6]、藤井久男[7]、池内浩基[8]、吉岡和彦[9]、板橋道朗[10]、渡邉聡明[11]、楠　正人[12]、橋本拓造[10]、辰巳健志[5]、内野　基[8]、河口貴昭[13]、高津典孝[14]、石黒　陽[15]、仲瀬裕志[16]、大宮美香[17]、平井郁仁[14]、池田圭祐[18]、山田哲弘[2]、松岡克善[19]、長沼　誠[19]、福地　工[20]、長堀正和[21]、渡辺　守[21]（東北大学大学院消化管再建医工学・分子病態外科学分野[1]、東邦大学医療センター佐倉病院内科[2]、東北大学大学院生体調節外科学分野[3]、東北労災病院大腸肛門外科[4]、横浜市民病院外科[5]、福岡大学筑紫病院外科[6]、奈良県立医科大学中央内視鏡・超音波部[7]、兵庫医科大学外科[8]、関西医科大学付属枚方病院外科[9]、東京女子医科大学第二外科[10]、帝京大学消化器外科[11]、三重大学消化管・小児外科学[12]、社会保険中央病院内科[13]、福岡大学消化器内科筑紫病院消化器内科[14]、弘前大学光学医療診療部[15]、京都大学消化器内科[16]、関西医科大学香里病院消化器内科[17]、福岡大学筑紫病院病理[18]、慶應義塾大学医学部消化器内科[19]、大阪済生会中津病院消化器内科[20]、東京医科歯科大学消化器病態学[21]）

炎症性腸疾患における血栓症発症の頻度および危険因子に関する多施設共同研究の実施状況

○藤谷幹浩[1]、安藤勝祥[1]、伊藤貴博[1]、稲場勇平[1]、上野伸展[1]、盛一健太郎[1]、前本篤男[2,3]、蘆田知史[2,3]、田邊裕貴[4]、高後　裕[1]（旭川医科大学内科学講座　消化器・血液腫瘍制御内科学分野[1]、旭川医科大学消化管再生修復医学講座[2]、札幌東徳州会病院IBDセンター[3]、国際医療福祉大学病院消化器内科[4]）

B-(4)-2　炎症性腸疾患にともなう感染症の現状とその対策（16:15～16:35）

総括　岡崎和一　関西医科大学　消化器・肝臓内科

我が国における炎症性腸疾患の急性増悪・再燃因子の前向き実態調査（特に感染症との関連性）

岡崎和一[1]、○大宮美香[1]、深田憲将[1]、佐々木誠人[2]、渡辺憲治[3]、大川清孝[4]、加賀谷尚史[5]、高添正和[6]、酒匂美奈子[6]、渡辺守[7]、長堀正和[7]、飯塚文瑛[8]、後藤秀実[9]、谷田諭史[9]、花井洋行[10]、飯田貴之[10]、平田一郎[11]、藤田浩史[11]、加藤　順[12]（関西医科大学内科学第三講座[1]、愛知医科大学消化器内科[2]、大阪市立大学消化器内科[3]、大阪市立十三市民病院[4]、金沢大学消化器内科[5]、社旗保険中央総合病院IBDセンター[6]、東京医科歯科大学消化器病態学[7]、東京女子医科大学IBDセンター[8]、名古屋市立大学消化器・代謝内科[9]、浜松南病院IBDセンター[10]、藤田保健衛生大学消化管内科[11]、和歌山県立医科大学第二内科[12]）

炎症性腸疾患におけるHBV感染の現状

坪内博仁[1,2]、○沼田政嗣[1]、森内昭博[1]、上村修司[1]、玉井　努[1]、船川慶太[1]、藤田　浩[1]、宇都浩文[1]、桶谷　眞[1]、井戸章雄[1]（鹿児島大学大学院 消化器疾患・生活習慣病学[1]、鹿児島市立病院[2]）

B-(4)-3　炎症性腸疾患と他臓器相関に関する臨床研究（16:35～16:55）

総括　岡崎和一　関西医科大学　消化器・肝臓内科

多施設共同観察研究　炎症性腸疾患に合併する自己免疫性膵炎の実態調査（最終報）

― 難治性膵疾患に関する調査研究班との共同研究 ―

○岡崎和一[1]、渡辺　守[2]、川　茂幸[3]、下瀬川　徹[4]（関西医科大学内科学第三講座[1]、東京医科歯科大学消化器病態学[2]、信州大学医学部内科学第二講座[3]、東北大学消化器内科[4]）

免疫修飾的治療下の炎症性腸疾患患者に対するインフルエンザワクチン接種の有効性の検討、中間報告

～「予防接種に関するワクチンの有効性・安全性等についての分析疫学研究：廣田班」との共同研究 ～

◯渡辺憲治[1]、松本紘子[1]、大藤さとこ[2]、萩原良恵[1]、山上博一[1]、荒川哲男[1]、廣田良夫[2]

(大阪市立大学大学院医学研究科消化器内科学[1]、公衆衛生学[2])

B-(4)-4　炎症性腸疾患患者の妊娠出産における現状とその対策 (16:55～17:10)

総括　三浦総一郎　防衛医科大学校内科学講座 (穂苅量太)

妊娠出産の転帰と治療内容に関する多施設共同研究の状況

三浦総一郎[1]、◯穂苅量太[1]、高本俊介[1]、渡辺知佳子[1]、長堀正和[2]、渡辺　守[2]、松岡克善[3]、長沼　誠[3]、日比紀文[4]、本谷　聡[5]、樋田信幸[6]、国崎玲子[7]、高橋宏和[7]、吉村直樹[8]、飯塚文瑛[9]、藤盛健二[10]、猿田雅之[11]、谷田諭史[12]、藤山佳秀[13]、内藤裕二[14]、渡辺憲治[15]、飯島英樹[16]、上野義隆[17]、田中信治[17]、石原俊治[18]、杉田　昭[19]、池上幸治[20]、松本主之[20]、仲瀬裕志[21]、岡崎和一[22]、石黒　陽[23]、松本吏弘[24]、　嵜山敏男[25]、小林清典[26]、横山　薫[26]、松井敏幸[27]、鶴身小都絵[27]、加賀谷尚史[28]、井上拓也[29] (順不同) (防衛医科大学校内科[1]、東京医科歯科大学消化器病態学[2]、慶應義塾大学医学部消化器内科[3]、北里大学北里研究所病院炎症性腸疾患先進治療センター[4]、札幌厚生病院 IBD センター[5]、兵庫医科大学内科学下部消化管科[6]、横浜市立大学消化器内科[7]、社会保険中央総合病院内科[8]、東京女子医科大学 IBD センター (消化器内科)[9]、埼玉医大消化器肝臓内科[10]、慈恵会医科大学付属病院消化器・肝臓内科[11]、名古屋市立大学病院消化器内科[12]、滋賀医科大学消化器内科[13]、京都府立医科大学消化器内科[14]、大阪市立大学病院消化器内科[15]、大阪大学医学部付属病院消化器内科[16]、広島大学病院内視鏡診療科[17]、島根医科大学消化器内科[18]、横浜市民病院外科[19]、岩手医科大学内科学講座消化器内科消化管分野[20]、京都大学消化器内科[21]、関西医大消化器肝臓内科[22]、弘前大学光学医療科[23]、さいたま医療センター消化器科[24]、鹿児島大学医学部付属病院消化器内科[25]、北里大学東病院消化器内科[26]、福岡大学筑紫病院消化器内科[27]、金沢大学附属病院消化器内科[28]、大阪医科大学消化器内科[29])

B-(4)-5　高齢及び小児期発症炎症性腸疾患患者の治療指針の必要性　(17:10 ～17:30)

総括　三浦総一郎　防衛医科大学校内科学講座 (穂苅量太)

高齢者炎症性腸疾患診療の現状把握 ― 前向き多施設共同研究の経過報告 ―

三浦総一郎[1]、◯高本俊介[1]、穂苅量太[1]、渡辺知佳子[1]、田中浩紀[2]、本谷　聡[2]、松本吏弘[3]、長堀正和[4]、渡辺　守[4]、松岡克善[5]、金井隆典[5]、小林　拓[6]、日比紀文[6]、横山　薫[7]、小林清典[7]、谷田諭史[8]、瀬戸山仁[9]、藤田　浩[9]、坪内博仁[9]、高橋晴彦[10]、松井敏幸[10]、加藤真吾[11] (順不同) (防衛医科大学校内科[1]、札幌厚生病院 IBD センター[2]、自治医科大学付属さいたま医療センター消化器科[3]、東京医科歯科大学消化器病態学[4]、慶應義塾大学医学部消化器内科[5]、北里大学北里研究所病院炎症性腸疾患先進治療センター[6]、北里大学東病院消化器内科[7]、名古屋市立大学病院消化器内科[8]、鹿児島大学医学部付属病院消化器内科[9]、福岡大学筑紫病院消化器内科[10]、埼玉医科大学総合医療センター消化器内科[11])

小児期発症炎症性腸疾患の治療に関する全国調査

◯清水俊明[1]、友政　剛[2]、田尻　仁[3]、国崎玲子[4]、石毛　崇[5]、山田寛之[6]、新井勝大[7]、大塚宜一[1]、余田　篤[8]、牛島高介[9]、青松友槻[8]、永田　智[10]、内田恵一[11]、竹内一夫[12]、穂刈量太[13]、三浦総一郎[13]、渡辺　守[14]

(順天堂大学医学部小児科[1]、パルこどもクリニック[2]、大阪府立急性期・総合医療センター小児医療センター[3]、横浜市立大学附属市民総合医療センター[4]、群馬大学大学院医学系研究科小児科学[5]、大阪府立母子センター消化器内分泌科[6]、国立成育医療研究センター消化器科[7]、大阪医科大学泌尿生殖発達医学講座小児科[8]、久留米大学医療センター小児科[9]、東京女子医科大学小児科[10]、三重大学医学部小児外科[11]、埼玉大学教育学部学校保健学講座[12]、防衛医科大学内科[13]、東京医科歯科大学消化器病態学[14])

事務局連絡

(17:30 終了予定)

懇親会 (17:30～)

Ⅰ．研究報告（続）

p-C）　基礎プロジェクト

C-(1)　診療に有用なバイオマーカー開発

C-(1)-1　免疫関連バイオマーカーの開発（9:00～9:30）

総括　竹田　潔　大阪大学大学院医学系研究科、千葉　勉　京都大学大学院医学研究科消化器内科学

虫垂リンパ組織の腸管恒常性維持における役割

○竹田　潔（大阪大学大学院医学系研究科免疫制御学）

クローン病における Th17 誘導性ミエロイド細胞の役割

○荻野崇之[1]、西村潤一[1]、香山尚子[2]、Soumik Barman[2]、植村　守[1]、畑　泰司[1]、竹政伊知朗[1]、水島恒和[1]、山本浩文[1]、土岐祐一郎[1]、森　正樹[1]、竹田　潔[2]（大阪大学大学院医学系研究科消化器外科[1]、大阪大学大学院医学系研究科免疫制御学[2]）

CXCR3 阻害剤の役割と治療応用

○石黒　陽[1,2]、櫻庭　裕丈[2]、蓮井　桂介[2]、平賀　寛人[2]、福田　眞作[2]
（国立病院機構弘前病院消化器血液内科[1]、弘前大学消化器血液内科[2]）

クローン病感受性遺伝子A20による腸炎惹起機構

○大島　茂、松沢　優、高原政宏、小林正典、仁部洋一、前屋舗千明、永石宇司、土屋輝一郎、岡本隆一、中村哲也、渡辺　守（東京医科歯科大学消化器病態学）

炎症性腸疾患の病態におけるリンパ管新生の関与炎症性腸疾患の病態におけるリンパ管新生の関与

○佐藤宏和[1]、成松和幸[1]、安武優一[1]、丸田紘一[1]、栗原千枝[1]、渡辺知佳子[1]、岡田義清[1]、碓井真吾[1]、冨田謙吾[1]、高本俊介[1]、永尾重昭[1]、穂苅量太[1]、三浦総一郎[2]（防衛医科大学校消化器内科[1]、防衛医科大学校[2]）

C-(1)-2　臨床的バイオマーカーの開発（9:30 ～9:54）

総括　日比紀文　北里大学北里研究所病院炎症性腸疾患先進治療センター

潰瘍性大腸炎に対する血球成分除去療法前後のケモカインプロファイリング

坪内博仁[1,2]、○上村修司[1]、小野陽平[1]、沼田政嗣[1]、瀬戸山仁[1]、藤田　浩[1]、井戸章雄[1]、嵜山敏男[3]、児玉眞由美[4]
（鹿児島大学大学院 消化器疾患・生活習慣病学[1]、鹿児島市立病院[2]、出水総合医療センター[3]、宮崎医療センター病院[4]）

腸炎における Serum - derived Hyaluronan - Associated Protein （SHAP）発現

佐々木誠人、○山口純治、野田久嗣、春日井邦夫（愛知医科大学消化器内科（消化管部門））

腸管炎症バイオマーカーとしての Fecal lipocalin の意義

千葉　勉[1]、仲瀬裕志[1]、○松浦　稔[1]、吉野琢哉[1]（京都大学医学部　消化器内科[1]）

CAP 治療効果予測因子としての温感と皮膚還流圧の上昇

○飯塚政弘[1,2]、衛藤　武[2]、相良志穂[1]、沼田友華[3]、柳原　悠[3]、熊谷　誠[3]（秋田赤十字病院附属あきた健康管理センター[1]、秋田赤十字病院消化器科[2]、秋田赤十字病院臨床工学課[3]）

C-(1)-3　疾患特異的バイオマーカーの開発（9:54～10:06）

（総括　坪内博仁　鹿児島大学大学院医歯学総合研究科寄附講座　HGF 組織修復・再生医療学講座）

総括　渡辺　守　東京医科歯科大学消化器病態学

IBD バイオマーカーLRG の臨床応用に向けて

○新﨑信一郎[1]、飯島英樹[1]、松岡克善[2]、金井隆典[2]、辻井正彦[1]、竹原徹郎[1]、本田宏美[3]、三嶋　隆[3]、仲　哲治[3]
（大阪大学大学院医学系研究科・消化器内科学[1]、慶應義塾大学内科[2]、医薬基盤研究所・免疫シグナルプロジェクト[3]）

クローン病特異的バイオマーカーの検討-多施設共同研究

○光山慶一[1]、 松井敏幸[2]、 金城福則[3]、 牧山和也[4]、 坪内博仁[5] (久留米大学医学部消化器内科 IBD センター[1]、福岡大学筑紫病院消化器科[2]、琉球大学医学部光学医療診療部[3]、社会医療法人春回会井上病院[4]、鹿児島大学医学部消化器・生活習慣病学[5])

C-(1)-4 腸内細菌関連バイオマーカーの開発 (10:06～10:30)

総括 藤山佳秀 滋賀医科大学消化器内科

クローン病腸内細菌叢の pyrosequence (Illumina) 解析

○藤山佳秀[1]、安藤 朗[2]、辻川知之[3]、馬場重樹[1]、西田淳史[1]、高橋憲一郎[2]、藤本剛英[2] (滋賀医科大学消化器内科[1]、滋賀医科大学大学院消化器免疫[2]、滋賀医科大学総合内科学[3])

大腸全摘術後回腸嚢腸内細菌叢と抗菌剤服用の影響

○福島浩平[1]、小森佑奈[1]、佐々木健吾[1]、斎藤 喬[1]、神山篤史[2]、渡辺和宏[2]、羽根田祥[2]、長尾宗紀[2]、鈴木秀幸[2]、舟山裕士[3]、高橋賢一[3]、佐々木巌[4] (東北大学大学院消化管再建医工学・分子病態外科学分野[1]、東北大学大学院生体調節外科学分野[2]、東北労災病院大腸肛門外科[3]、みやぎ健診プラザ[4])

UC 患者腸液中の CMV-DNA 検出の検討

金城福則[1]、○伊良波 淳[1]、金城 徹[1]、岸本一人[2]、外間 昭[2]、上原綾子[2]、金城武士[2]、藤田次郎[2]
(琉球大学医学部附属病院光学医療診療部[1]、琉球大学医学部附属病院第一内科[2])

乳酸菌由来ポリリン酸を用いた新規炎症性腸疾患治療薬の臨床応用へ向けた開発研究

○藤谷幹浩[1]、上野伸展[1]、稲場勇平[1]、盛一健太郎[1]、前本篤男[2,3]、蘆田知史[2,3]、田邊裕貴[4]、高後 裕[1] (旭川医科大学内科学講座 消化器・血液腫瘍制御内科学分野[1]、旭川医科大学消化管再生修復医学講座[2]、札幌東徳洲会病院 IBD センター[3]、国際医療福祉大学病院消化器内科[4])

C-(1)-5 炎症による発癌バイオマーカーの開発 (10:30～11:00)

総括 味岡洋一 新潟大学院医歯学総合研究科分子診断病理学分野

潰瘍性大腸炎の炎症性発癌と DNA 損傷応答

○谷 優佑[1]、味岡洋一[1]、渡辺佳織里[1]、若井俊文[2]、山口尚之[1] (新潟大学大学院医歯学総合研究科分子診断病理学分野[1]、新潟大学大学院医歯学総合研究科消化器一般外科分野[2])

潰瘍性大腸炎の炎症粘膜における遺伝子メチル化の検討―臨床像との関連も含めて―

○田原智満、平田一郎、中野尚子、長坂光夫、中川義仁、大宮直木 (藤田保健衛生大学消化管内科)

オルガノイド培養系の確立と腸炎関連発がん機構の解明への活用

○南木康作[1]、佐藤俊朗[1]、中里圭宏[1]、武下達矢[1]、松岡克善[1]、矢島知治[1]、久松理一[1]、井上 詠[2]、岩男 泰[2]、長沼 誠[3]、緒方晴彦[3]、藤井正幸[4]、渡邉聡明[4]、金井隆典[1] (慶應義塾大学医学部内科学教室消化器内科[1]、慶應義塾大学医学部予防医療センター[2]、慶應義塾大学医学部内視鏡センター[3]、東京大学医学部腫瘍外科[4])

炎症性発癌におけるMFG-Eの役割

石原俊治、○楠 龍策、多田育賢、園山浩紀、岡 明彦、福廻暢彦、大嶋直樹、森山一郎、結城崇史、川島耕作、木下芳一
(島根大学医学部内科学講座第二)

粘液産生がんにおけるAtoh1発現の意義

○土屋 輝一郎、福島啓太、加納嘉人、堀田伸勝、日比谷秀爾、林 亮平、大島 茂、岡本隆一、永石宇司、中村哲也、渡辺 守 (東京医科歯科大学消化器病態学)

C-(2)　粘膜修復機構解析と治療応用　(11:00～11:18)

総括　有村　佳昭　札幌医科大学消化器・免疫・リウマチ内科学

MSC依存性細胞増殖の機序

◯一色裕之、小野寺馨、永石歓和、今井浩三、篠村恭久、有村佳昭（札幌医科大学消化器・免疫・リウマチ内科学）

Wnt5a peptideによる大腸上皮の修復

◯内藤裕二[1]、高木智久[1]、◯内山和彦[1]（京都府立医科大学消化器内科）

腸管上皮幹細胞培養とその臨床応用技術開発

◯中村哲也、水谷知裕、福田将義、野崎賢吾、渡辺　守（東京医科歯科大学消化器病態学）

Ⅱ．国立保健医療科学院挨拶

国立保健医療科学院健康危機管理研究部上席主任研究官　武村　真治　先生　(11:20～11:30)

事務局連絡

閉会挨拶

(11:40終了予定)

Ⅶ.　研究成果の別刷

Please cite this article in press as: Fordham et al., Transplantation of Expanded Fetal Intestinal Progenitors Contributes to Colon Regeneration after Injury, Cell Stem Cell (2013), http://dx.doi.org/10.1016/j.stem.2013.09.015

Cell Stem Cell
Article

Transplantation of Expanded Fetal Intestinal Progenitors Contributes to Colon Regeneration after Injury

Robert P. Fordham,[1,2,6] Shiro Yui,[3,4,6] Nicholas R.F. Hannan,[1,2] Christoffer Soendergaard,[5] Alison Madgwick,[1] Pawel J. Schweiger,[3] Ole H. Nielsen,[5] Ludovic Vallier,[1,2] Roger A. Pedersen,[1,2] Tetsuya Nakamura,[4] Mamoru Watanabe,[4] and Kim B. Jensen[1,3,*]

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http://dx.doi.org/10.1016/j.stem.2013.09.015

SUMMARY

Regeneration and homeostasis in the adult intestinal epithelium is driven by proliferative resident stem cells, whose functional properties during organismal development are largely unknown. Here, we show that human and mouse fetal intestine contains proliferative, immature progenitors, which can be expanded in vitro as Fetal Enterospheres (FEnS). A highly similar progenitor population can be established during intestinal differentiation of human induced pluripotent stem cells. Established cultures of mouse fetal intestinal progenitors express lower levels of Lgr5 than mature progenitors and propagate in the presence of the Wnt antagonist Dkk1, and new cultures can be induced to form mature intestinal organoids by exposure to Wnt3a. Following transplantation in a colonic injury model, FEnS contribute to regeneration of colonic epithelium by forming epithelial crypt-like structures expressing region-specific differentiation markers. This work provides insight into mechanisms underlying development of the mammalian intestine and points to future opportunities for patient-specific regeneration of the digestive tract.

INTRODUCTION

Fertilization of the oocyte initiates a series of events that, following gastrulation, leads to organ formation in the developing fetus. During this process, pluripotent stem cells progressively lose potential as the early embryo is patterned along its axes and organ structures are specified. Tissue-specific programs subsequently direct the formation and maturation of adult organs, which are maintained throughout life by stem cells with tissue-restricted lineage potential. It remains unclear whether transitory stem cell states exist in the embryo, responsible for tissue maturation, or whether maturation is achieved via adult tissue-specific stem cells in the fetal tissue. Understanding the process of tissue maturation in vivo has implications for the directed differentiation of pluripotent cells into functionally mature tissue types (Zorn and Wells, 2009).

The intestinal epithelium is continuously replenished by resident stem cells. The mature mammalian small intestine is a tube-like structure with an inner epithelial lining facing the lumen. This layer is organized into differentiated villi protruding into the lumen and proliferative crypt compartments invaginated into the underlying mesenchyme. Intestinal Stem Cells (ISCs) reside at the crypt base and give rise to all the differentiated cell types (Barker et al., 2007, 2012). Development of the small intestine follows a specific pattern. Villus formation in humans begins around the ninth week of gestation and embryonic day 15 (E15) in mouse. In the human, crypt formation occurs before birth, whereas in the mouse this happens during the first 2 postnatal weeks (Montgomery et al., 1999; Spence et al., 2011a). Beyond these morphological rearrangements, the mechanisms of initial intestinal lineage differentiation and functional maturation are less well characterized. Despite temporal differences in the ontogeny of the small intestine between human and mouse, the overall process of development is identical, making the mouse an accessible model to interrogate the process of human intestinal maturation.

Our understanding of the mature intestine has been accelerated by the establishment of culture conditions for long-term maintenance of adult mouse and human intestinal epithelium in vitro (Jung et al., 2011; Sato et al., 2009, 2011a). In this system, single ISCs or dissociated crypt fragments are embedded in Matrigel where they exhibit self-organization into "mini-guts." Here we describe the identification of proliferative progenitors captured in the human fetal intestine and during intestinal differentiation of human induced pluripotent stem cells (hiPSCs). This

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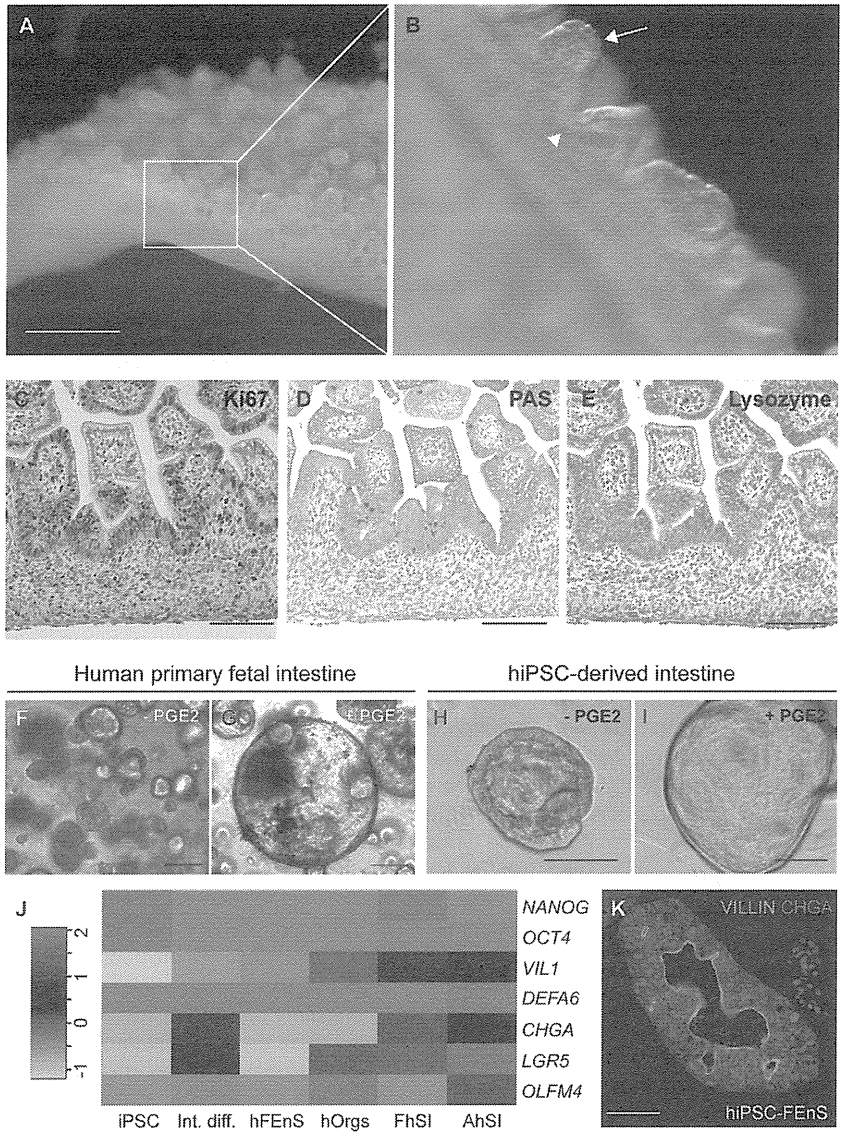

Figure 1. Derivation of Immature Intestinal Progenitors from Human Fetal and Pluripotent Cells

(A) Whole mount of human gestational week 10 small intestine.

(B) Higher magnification of villi (arrow) and intervillus regions (arrowhead) in (A).

(C–E) Immunohistochemistry analysis for Ki67 (C), PAS staining (D), and Lysozyme (E) in week 10 human small intestine.

(F and G) Spheroid cultures from week 10 human small intestinal epithelium, grown with (G) and without (F) prostaglandin E2 (PGE2) (2.5 μM).

(H and I) Intestinal tissue derived from directed differentiation of human induced pluripotent stem cells (hiPSCs), cultured with (I) and without (H) PGE2.

(J) Relative expression levels of intestinal lineage markers in material from undifferentiated human induced pluripotent stem cells (hiPSC), iPSC-derived intestine (Int. diff.), human primary fetal enterospheres (hFEnS), human adult organoids (hOrgs), primary fetal human small intestine (FhSI), and primary adult human small intestine (AhSI). Red and green colors reflect increased and decreased deviation from the mean, respectively.

(K) Detection of VILLIN (green) and CHGA (red) in hiPSC-FEnS.

The scale bars represent 2 mm in (A) and 100 μm in (C)–(E) and (K). See also Figure S1 and Table S1.

is recapitulated in murine tissues, where fetal progenitors can transition spontaneously and by Wnt induction into an adult state. Finally, we present evidence that fetal progenitors can contribute to the regeneration of adult colonic epithelium in vivo, as proof of principle that developmentally immature cells have clinical potential.

RESULTS

Fetal Human Intestinal Epithelium Can Be Propagated Long-Term In Vitro as Fetal Enterospheres

Previous studies have described the establishment of organoid cultures from mature human gut epithelium (Jung et al., 2011; Sato et al., 2011a). To investigate the in vitro potential of immature gut epithelium, we analyzed human fetal intestinal tissue around gestational week 10. At this stage, crypts have not formed and the human intestine consists of a series of undulating villi, with proliferation localized primarily to the intervillus regions (Figures 1A–1C). Here a subset of cells is weakly positive for Periodic Acid Schiff's (PAS), though they do not have the mature morphology of goblet cells and there are no detectable Lysozyme^{+ve} Paneth cells (Figures 1D and 1E). The reduced level of secretory differentiation was confirmed at the transcriptional level (Figure 1J).

Fetal human intestinal tissue at around gestational week 10 was dissected and dissociated epithelial fragments were seeded in Matrigel. The conditions used for propagation of adult murine organoids (EGF, Noggin, and R-spondin1 [ENR]) caused the growth of small granular spheres that could not be maintained long-term without the addition of prostaglandin-E2 (PGE2) (Figures 1F and 1G). We term these human Fetal Enterospheres (hFEnS). hFEnS are highly proliferative and can be passaged repeatedly by mechanical dissociation for over 2 months with no spontaneous transition into budding organoids during this time.

Intestinal Tissue from Human Pluripotent Cells Has Fetal Characteristics

Human induced pluripotent stem cells (hiPSCs) can be differentiated into intestinal epithelium (Spence et al., 2011b). We set out to determine whether hiPSC-derived intestinal tissue transitions through a fetal state. Using a chemically defined protocol, PSCs were directed toward definitive endoderm (DE) and further

patterned into posterior DE (Hannan et al., 2013). Raised aggregates of cells forming from the sheet of posteriorized endoderm were transferred as small clumps to Matrigel (Figure S1A available online). Again PGE2 facilitated the formation of larger cystic epithelial spheroids, morphologically analogous to primary hiFEnS (Figures 1H and 1I). These structures were maintained for over 2 months, through repeated passaging. In both cases PGE2 provides a pro-proliferative signal that drives the growth of spherical structures. hiPSC-FEnS also require low levels of Wnt3a to support growth, suggesting that although morphologically alike, they possess slightly different properties. Expression analysis verifies the immature nature of human FEnS and hiPSC-FEnS when compared to human adult organoids as well as fetal and adult intestine (Figures 1J and S1B). iPSC-derived FEnS had Villin present at the apical cell membrane in the spherical structure, and its immature nature is further supported by the lack of secretory Chromogranin-A^{+ve} cells (Figure 1K).

Establishment of FEnS from Immature Mouse Intestine

We reasoned that development of the mouse intestine would provide an accessible model system to interrogate intestinal maturation more closely. The mouse intestine at embryonic day 16 (E16) resembles the human intestine at around 10 gestational weeks with high proliferation in the intervillus regions and scattered immature goblet cells (Figures 2A, S2A, S2B, S2E, and S2F). By postnatal week 2, mature crypts are forming (Figures 2B, S2C, and S2D) and mature Lysozyme^{+ve} Paneth cells can now be detected in the proliferative zones (Figures S2G and S2H). The appearance of secretory cells is also evident by expression analysis during the course of intestinal development (Figure 2C).

To investigate whether fetal murine intestine contains equivalent FEnS progenitors, we seeded epithelial cells from the proximal half of the small intestine. During a developmental time course, we observed that FEnS form exclusively up to P2, whereas organoids are formed from P15 and onward (Figures 2D–2F and 2I). Interestingly, analysis of material from P2 to P15 illustrates the formation of both FEnS and organoids with an increasing fraction of the latter (Figures 2G–2I). Murine FEnS (mFEnS) are morphologically indistinguishable from hFEnS and can be expanded through fortnightly passaging for at least 2 years (Passage n $\approx$ 100). During their serial passaging we observe no spontaneous maturation or morphological and karyotypic alterations (Figure 2J). Although PGE2 is not required for maintenance of mFEnS, it does provide a pro-proliferative effect independent of Wnt signaling (Figure S2I). As has been reported for the adult colonic cultures, this is most likely via cAMP-mediated block of anoikis and stimulation of MAP kinase signaling (Jung et al., 2011). Established mFEnS can grow without R-spondin1 and in the presence of the natural Wnt antagonist DKK1, Porcupine inhibitor (which inhibits Wnt secretion), and tankyrase inhibitor (which stabilizes the Axin2/APC complex responsible for degradation of β-catenin), hereby demonstrating that FEnS can be maintained independently of Wnt signaling (Figures S2J and S2K). This distinguishes them from adult organoids.

Characterization of mFEnS revealed that they consist of a polarized epithelium with Villin localized to the apical surface, similar to the small intestine (Figures 2K and 2L). Moreover, FEnS phenocopy the differentiation patterns of the immature epithelium as there are no detectable secretory cell markers at both the protein and RNA level and reduced expression of adult stem cell markers (Figures 2M–2P and S3A). BrdU incorporation analysis showed that proliferative cells in mFEnS are scattered across the whole surface, whereas proliferative zones in organoids are restricted to the crypt domains (Figures 2Q and 2R). The overall morphology and growth of FEnS as spheres are reminiscent of that reported for organoids that form as a result of augmented Wnt signaling following loss of APC (Sato et al., 2011b). However, expression analysis demonstrates distinct expression patterns between FEnS and APCnull organoids (Figure S3B). In particular, it is clear that loss of APC causes increased levels of adult stem cell markers, whereas these are generally reduced in the fetal state (Figure S3B). In summary, this demonstrates that progenitors within the fetal small intestine have a unique behavior that sets them aside from both normal and cancerous adult stem cells.

In Vitro Maturation of Fetal Enteric Progenitors

Intestinal maturation in vivo has been proposed to follow a wave from proximal to distal sites (Spence et al., 2011a). To assess the positional effect along the length of the small intestine, we analyzed the regional differences in in vitro growth potential at postnatal day 2 (Figure 3A). Contrary to expectations, FEnS formed from proximal tissue, whereas more distal tissues formed organoids (Figures 3A and 3B). Gene expression analysis showed that the ability to form organoids correlates with increased levels of *Lgr5* and *Axin2* (Figure 3C). Analysis of the cultured material from the proximal and mid regions of the small intestine shows variable but comparable expression of Wnt target genes, suggesting that FEnS can respond to Wnt stimulation and that this represents a transitory and dynamic cellular state (Figure 3D). In line with the observed adult stem cell behavior, the distal part of the small intestine expresses higher levels of secretory lineage markers, which are characteristic of the adult small intestine, and contains a greater number of *Ulex europaeus* agglutinin I (UEA-I) reactive secretory cells (Figures 3C, 3E–3E″, and 3F). This further supports a distal to proximal wave of tissue maturation.

In the mature intestine, Lgr5 marks ISCs, and single sorted Lgr5^{+ve} cells give rise to adult organoids (Barker et al., 2007; Sato et al., 2009). In the immature intestine Lgr5 is expressed by cells in the intervillus regions (Figure 4A). We hypothesized that Lgr5 expression defines progenitors permissive for transitioning into the adult state. In line with this, Lgr5-GFP^{+ve} cells sorted from neonatal intestinal epithelium form organoids in vitro, whereas FEnS are formed from cells in the Lgr5-GFP^{-ve} population (Figures 4B–4E). It is impossible to assess whether organoids form exclusively from Lgr5^{+ve} cells, as a large proportion of Lgr5-expressing cells in the Lgr5 knockin model are EGFP^{-ve} due to the mosaic nature of the mouse model.

To assess the relationship between organoids and FEnS, we analyzed samples from P2. Approximately one-half of the structures grow in a manner indistinguishable from fetal tissues (Figure S4A, Movie S1), whereas the rest followed a distinct pattern indicative of spontaneous differentiation (Figure S4B, Movie S2). All structures grow exponentially for around 7 days. At this point some structures collapse and start to form budding

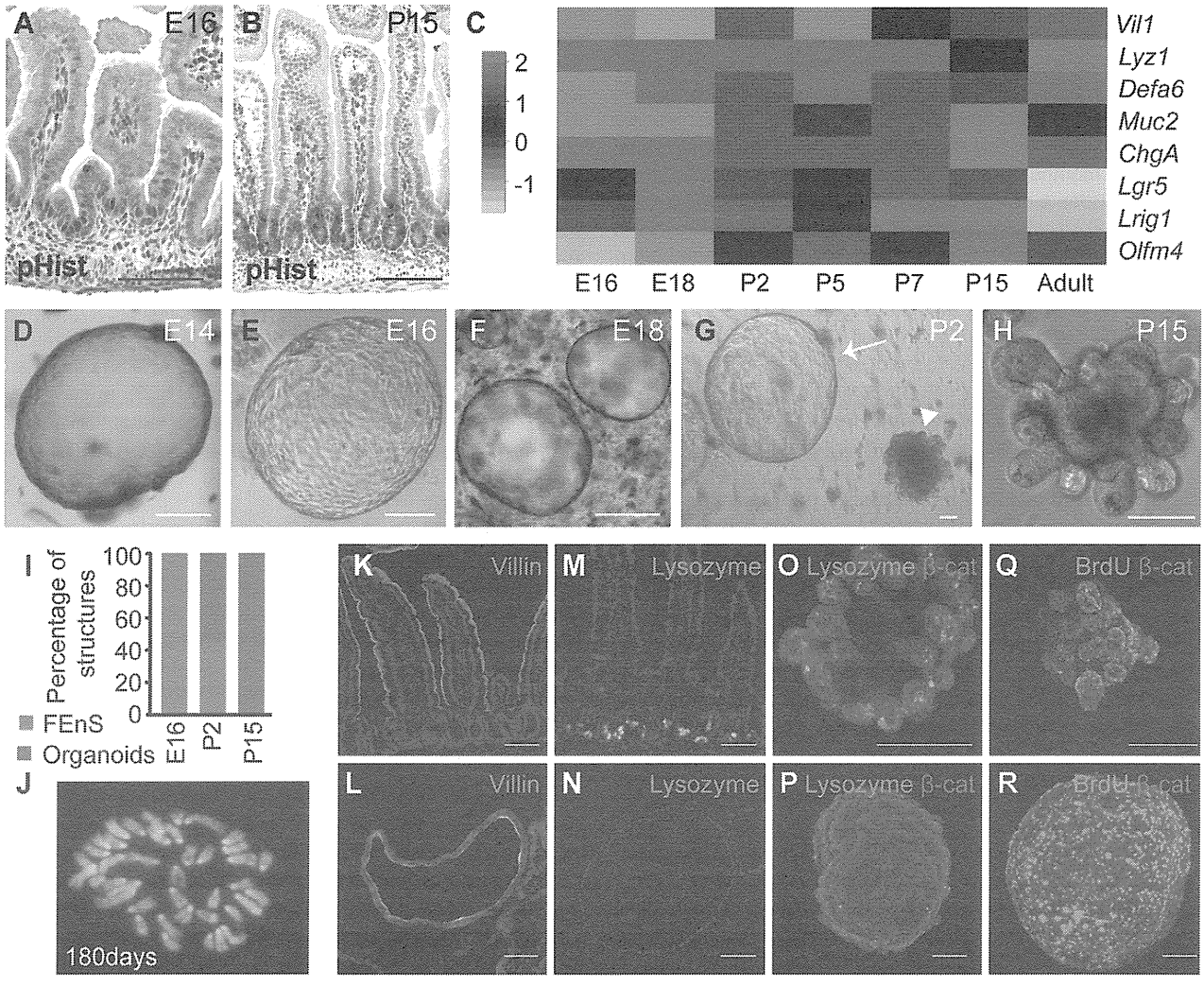

Figure 2. Establishment of mFEnS from Immature Mouse Intestine

(A and B) Immunohistochemistry analysis for Phospho-Histone-H3 (pHist) on sections of small intestine from E16 mice (A) and P15 mice (B).

(C) Relative expression levels of intestinal lineage markers in tissue isolated from proximal murine intestine at increasing developmental age from E16 to adult. Red and green colors reflect increased and decreased deviation from the mean, respectively.

(D–H) Representative images of in vitro structures derived from E14 to P15. The arrow and arrowhead in (G) indicate an FEnS and an organoid, respectively.

(I) Relative proportions of FEnS and organoids present after 2 weeks from E16, P2, and P15 tissues.

(J) Metaphase spread of a cell at day 180 shows a normal karyotype (n = 15).

(K and L) Detection of apical villin expression (green) in adult small intestine (K) and mFEnS (L).

(M–P) Lysozyme expression in adult small intestine (M), cross sections of mFEnS (N), and whole-mount organoids and mFEnS (O and P).

(Q and R) BrdU incorporation analysis in whole mounts of organoids and FEnS (green). β-catenin (red) is used as a counterstain.

The scale bars represent 100 μm. E, embryonic day; P, postnatal day; adult, >3 weeks postnatal. See also Figures S2 and S3.

protrusions from the surface (Figure S4B). After passaging, these P2 organoids become R-spondin1 dependent and identical to structures obtained from more mature intestinal tissue (Figure S4C, Movie S3).

Since *Lgr5* and *Axin2* are both Wnt target genes, and given the dynamic regional expression correlating with organoid formation (Figure 3D), we investigated whether Wnt3a can induce intestinal maturation in vitro. Stimulation of cells from E16 proximal intestine, which normally only form FEnS, promoted the transition into budding organoids in a proportion of the forming structures (Figure S4D). This effect is enhanced upon passaging and the forming organoids can subsequently be maintained without exogenous Wnt in an R-spondin1-dependent manner (Figure S4Dix). Continued culture of organoids with high levels of exogenous Wnt3a produced the cystic morphology previously described for Wnt overactivity in adult cultures (Sato et al., 2011b; Figures S4vi and S4vii). In contrast, FEnS could not be induced to transit to an adult state with Wnt3a (Figures S4iv and S4v). The observed Wnt-stimulated maturation of FEnS to organoids is associated with the expected upregulation of secretory lineage markers (Figure S4E). It is clear that FEnS respond to Wnt stimulation, as *Lgr5* and *Axin2* expression is elevated compared to

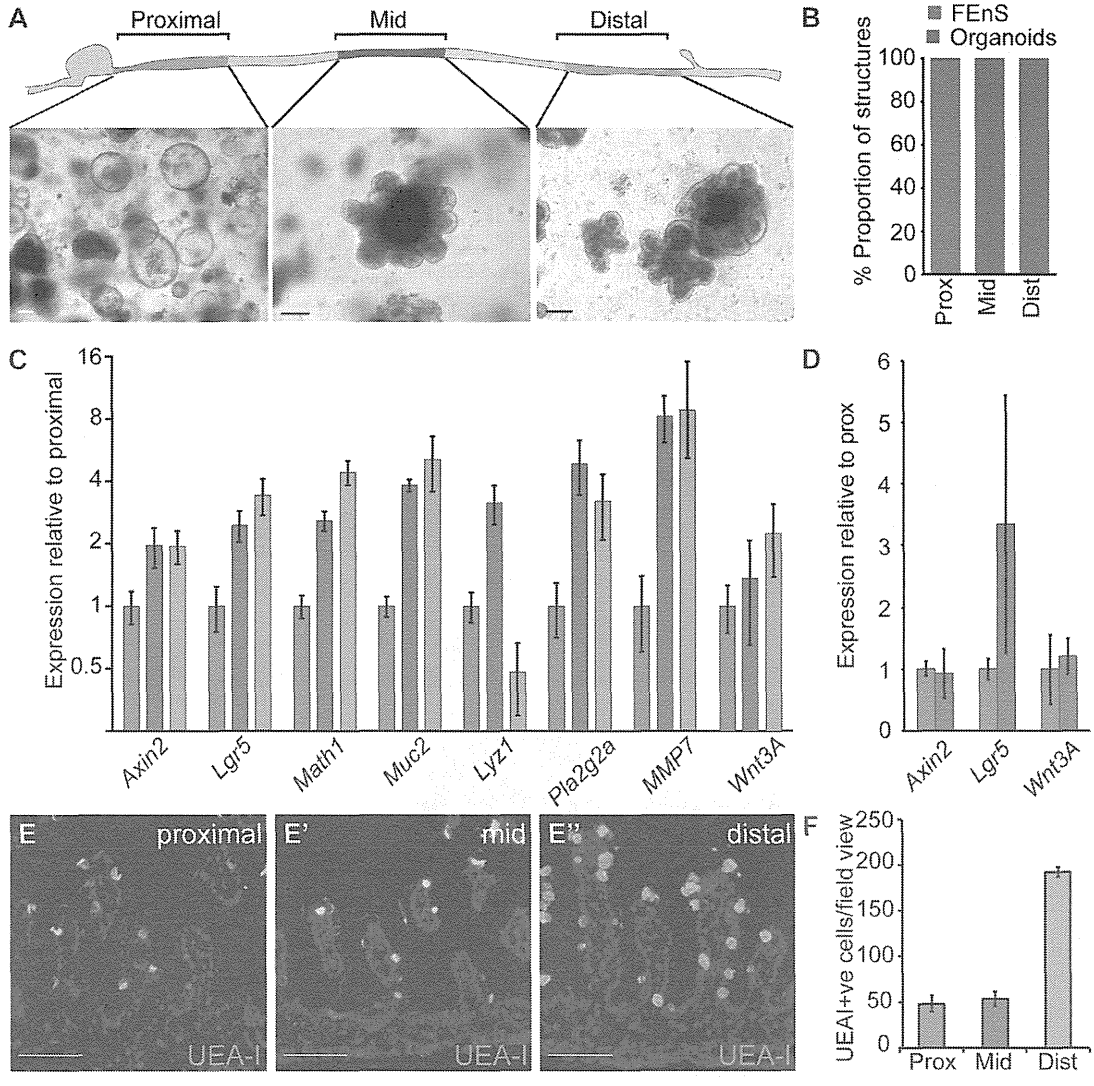

Figure 3. Adult Stem Cell Behavior Follows a Caudal to Rostral Pattern

(A) Schematic diagram of the Proximal, Mid, and Distal parts of the small intestine and the representative images of cultures derived at P2.

(B) Relative proportion of FEnS and organoids in the different sections of the small intestine.

(C) Expression analysis in material isolated from Proximal, Mid, and Distal regions. Data represent the mean, and the error bars, the SEM (n = 3). Data are expressed relative to Proximal, on a Log_2 scale.

(D) Expression analysis of cultures from proximal and mid intestine enriched for FEnS and organoids, respectively. Data represent the mean, and the error bars, the SEM (n = 3), and are normalized to proximal cultures.

(E) Detection of cells of the secretory lineage based on binding of *Ulex europaeus* agglutinin I (UEA-I) in the proximal, mid, and distal small intestine.

(F) Quantification of UEA-I^{+ve} cells. Data represent the mean, and the error bars, the SEM (n = 3).

The scale bars represent 100 μm.

established cultures and also newly transitory organoids (Figure S4E); however, the signal is insufficient to induce maturation.

In order to further probe the functional significance of Wnt in the transition from a fetal to an adult phenotype, epithelial cells were isolated from P2 proximal small intestine. Because a proportion of FEnS at this stage naturally transition to the adult organoids, it is possible to investigate the importance of Wnt signaling in the establishment of both organoids and FEnS, as well as the transition between the two states. Epithelial cells were isolated from the Lgr5-reporter model in order to visualize Lgr5 expression. Whenever we observe high Lgr5-EGFP expression, this is in association with structures that are beginning to transition into the adult state. In medium supplemented with ENR, $EGFP^{+ve}$ cells can be found either in mature crypt domains (Figures 4F and 4F′) or in regions with columnar morphology (Figures 4G and 4G′), whereas FEnS structures are seemingly $EGFP^{-}$ve or dim (Figures 4H and 4H′). The addition of Wnt increases the number of formed organoids (Figure 4N) and the regions of Lgr5 expression in the developing structures. This varies from single positive buds to extensive regions of Lgr5-$EGFP^{+ve}$

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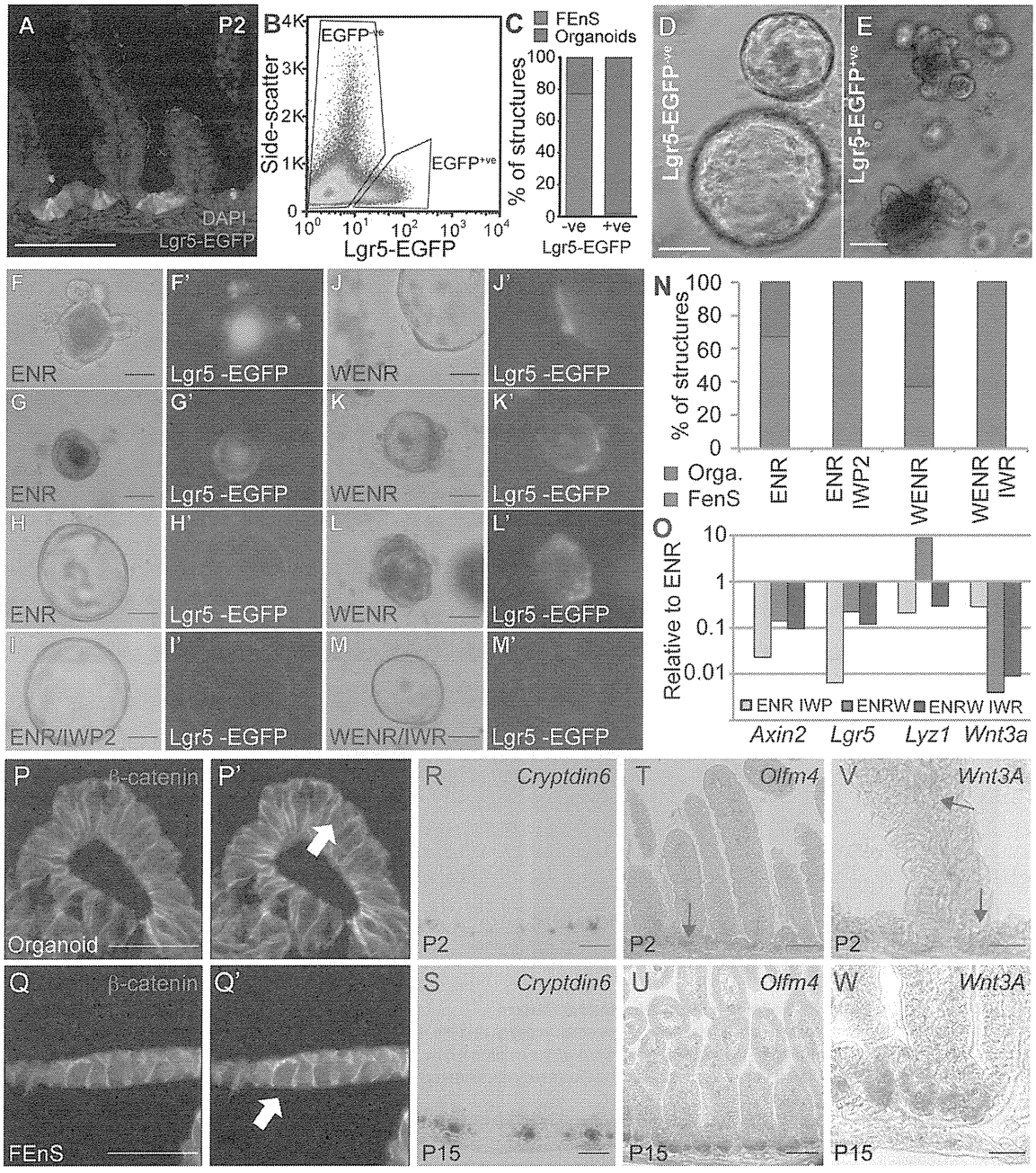

Figure 4. In Vitro Maturation of Fetal Enteric Progenitors Is Associated with Lgr5 Expression and Wnt Signaling

(A) Detection of Lgr5-EGFP at P2 from Lgr5-EGFP-ires-CreERT2 mice.

(B) Isolation of Lgr5-GFP^{+ve} and Lgr5-GFP^{-ve} epithelial cells from P2 small intestine by flow cytometry.

(C) Quantification of proportion of FEnS and organoids formed in vitro from Lgr5-GFP^{-ve} and Lgr5-GFP^{+ve} neonatal intestinal epithelial cells.

(D and E) Representative images of structures formed in vitro from Lgr5-GFP^{-ve} and Lgr5-GFP^{+ve} neonatal intestinal epithelial cells.

(F–M) Representative images of FEnS and organoids derived from Lgr5-EGFP-ires-CreERT2 mice and cultured in the presence of EGF, Noggin, and R-spondin1 (ENR), ENR and the porcupine inhibitor IWP2 (ENR/IWP2), ENR and Wnt3a (WENR), or WENR in the presence of the tankyrase inhibitor IWR (WENR/IWR). (F′)–(M′) show grayscale images of EGFP in the derived structures.

(N) Quantification of proportion of FEnS and organoids formed in the different treatment groups (ENR: 18/8; ENR/IWP2: 26/0; WENR: 21/30; WENR/IWR: 33/0). Two-tailed Fisher's exact test shows significant difference between ENR and ENR/IWP2 ($p = 0.0042$), ENR and WENR ($p = 0.0297$), and WENR and WENR/IWR ($p < 0.0001$).

(O) Expression analysis of the different treatment groups normalized to the ENR condition. Data represent the mean ($n = 2$).

(P and Q) Detection of β-catenin (green) in organoids and FEnS. Arrows indicate cells with nuclear localization of β-catenin suggestive of active signaling.

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cells (Figures 4J–4L′). The transition and Lgr5 expression is blocked by the addition of porcupine inhibitor to ENR-supplemented cultures (Figures 4I, 4I′, and 4N) and by the addition of the tankyrase inhibitor IWR-1 to cells cultured in the presence of ENR and Wnt (Figures 4M, 4M′, and 4N). Importantly, these inhibitors do not preclude the formation of FEnS. The maturation is reflected at the RNA level, where Wnt induces a robust increase in the Paneth cell marker Lysozyme. However, it is also clear that FEnS in early cultures express endogenous *Wnt3a*, which drives both *Axin2* and *Lgr5* expression within the fetal population of cells (Figure 4O). In line with the elevated expression of *Axin2* and *Lgr5*, β-catenin can be observed in the nucleus of cells in FEnS as well as in the formed organoids (Figures 4P and 4Q).

In vivo tissue maturation correlates with the emergence of secretory Paneth cells, which has been identified as the major source of epithelial Wnt secretion in the intestinal epithelium (Sato et al., 2011b; Farin et al., 2012). Although mature Lysozyme^{-ve} Paneth cells cannot be observed until postnatal week 2 (Figure sS2E–S2H), these are preceded by immature secretory cells, which can be detected based on *Cryptdin6* expression (Wong et al., 2012). Assessment of tissues from P2 and P15 demonstrates that *Cryptdin6*-expressing cells can be detected as early as P2 (Figures 4R and 4S). This correlates with the appearance of cells that are weakly positive for the stem cell marker *Olfm4* as well as *Wnt3a* within the bottom of the intervillus regions (Figures 4T–4W). This provides an epithelial source of Wnt3a that can drive tissue maturation.

In summary, this demonstrates that exogenous Wnt induces elevated focal Lgr5 upregulation in the fetal state and that maturation proceeds from these Lgr5 expression domains. Expression of *Wnt3a* can be detected in proliferative intervillus regions as the tissue proceeds into its adult state, suggesting that Wnt induction in vivo correlates with tissue maturation.

Regeneration of Adult Colonic Epithelium from mFEnS

To assess the differentiation potential of immature intestinal progenitors and whether they represent a transplantable source, EGFP^{+ve} established mFEnS were injected under the renal capsule of mice (n = 8). In all cases at analysis, EGFP FEnS cells had either not proliferated or were not detectable. To test a more physiologically relevant approach, we transplanted EGFP FEnS into a chemically induced colonic injury model, where the repair process is associated with endogenous activation of Wnt signaling (Figure 5A; Yui et al., 2012; Koch et al., 2011). Within 3 hr after the first transplantation, FEnS-derived cells attached to ulcerated regions in the distal colon and were subsequently maintained long-term (Figures 5B and S5A–S5H). Initially, cells engrafted as a single-layered epithelium on top of the denuded lamina propria (Figures S5I–S5J). Three days following transplantation, grafted regions migrated downward into the underlying mesenchyme. Here they formed epithelial "pockets" with a central lumen and Ki67^{+ve} cells distributed along the length (Figures 5C, S5K, and S5L). One week after the second transplantation, engrafted cells formed epithelial crypt-like structures. These fetal-derived cells, although refractory to maturation in vitro, adapt to the colonic tissues, with subsets of cells differentiating appropriately into Mucin-2^{+ve} and PAS^{+ve} goblet cells and starting to express carbonic anhydrase-II, a specific marker of colonic tissue. None of this was detected in FEnS (Figures 5C, S5L, S5N, and S5P–S5R). Importantly, the grafted material did not express markers normally associated with the small intestine such as Lysozyme and alkaline-phosphatase (Figures S5R–S5T). Fetal-derived colonic crypts persisted at 1.5 months after transplantation, with continued evidence for proper differentiation and proliferation (Figures 5C, S5O, and S5P). Thus, immature enteric progenitors represent a transplantable source of cells with the capacity to differentiate in vivo.

DISCUSSION

In this study, we reveal the existence of a transitory population of progenitors present during the intestinal growth phase in both human and murine tissues. Moreover, a population of cells with similar characteristics can be obtained from pluripotent stem cells. This population is characterized by distinct proliferative and differentiation potential and reduced in vitro growth factor requirements compared to progenitors in the adult intestinal epithelium. Transition of fetal enteric progenitors into an adult state can be induced in vitro via stimulation with high levels of Wnt or alternatively by transplantation in vivo into an injury model. These cells are a valuable asset for understanding tissue maturation and an attractive source of transplantable progenitors for regenerative therapies.

Studies of human organ development are complicated by the availability of material. We provide evidence that mouse and human fetal intestine contain an immature population of epithelial progenitors and that similar immature cells can be obtained from hPSCs. Here the immature progenitors represent a transitory population of cells. Interestingly, many differentiation protocols from PSCs result in cells with a stable immature phenotype (Meyer et al., 2009; Nicholas et al., 2013). Based on our results this is not necessarily a tissue culture artifact but rather a result of the in vitro stabilization of an otherwise transitory state in vivo. It is however clear that it is not straightforward to extrapolate growth factor requirements from mouse to human cells as has been reported for their adult counterparts (Jung et al., 2011; Sato et al., 2009, 2011a).

Intestinal maturation has been proposed to follow a rostral-to-caudal (proximal-to-distal) wave (Spence et al., 2011a). We observe that FEnS form from the proximal region and organoids from the distal region, indicating that maturation in actual fact proceeds in the opposite direction. This is correlated with the expression pattern of markers of the mature secretory lineage and correlates with the observation that Lgr5 expression is associated with progenitors in a transitory competent state. These spatial and temporal observations are in agreement with previous work showing that Lgr5 gene expression is higher in the ileum than in the duodenum at E18.5 (Garcia et al., 2009). It

(R–W) In situ hybridization for *Cryptdin6*, *Olfm4*, and *Wnt3a* in tissue from P2 and P15. Arrows in (S) and (U) indicate regions of Olfm4 and Wnt3a expression, respectively.

The scale bars represent 50 μm (F, J, K–L, P–Q, and V–W) or 100 μm (A, D–E, G–I, M, and R–U). Cells are counterstained with DAPI (blue) in (A), (O), and (P). See also Figure S4 and Movie S1, Movie S2, and Movie S3.

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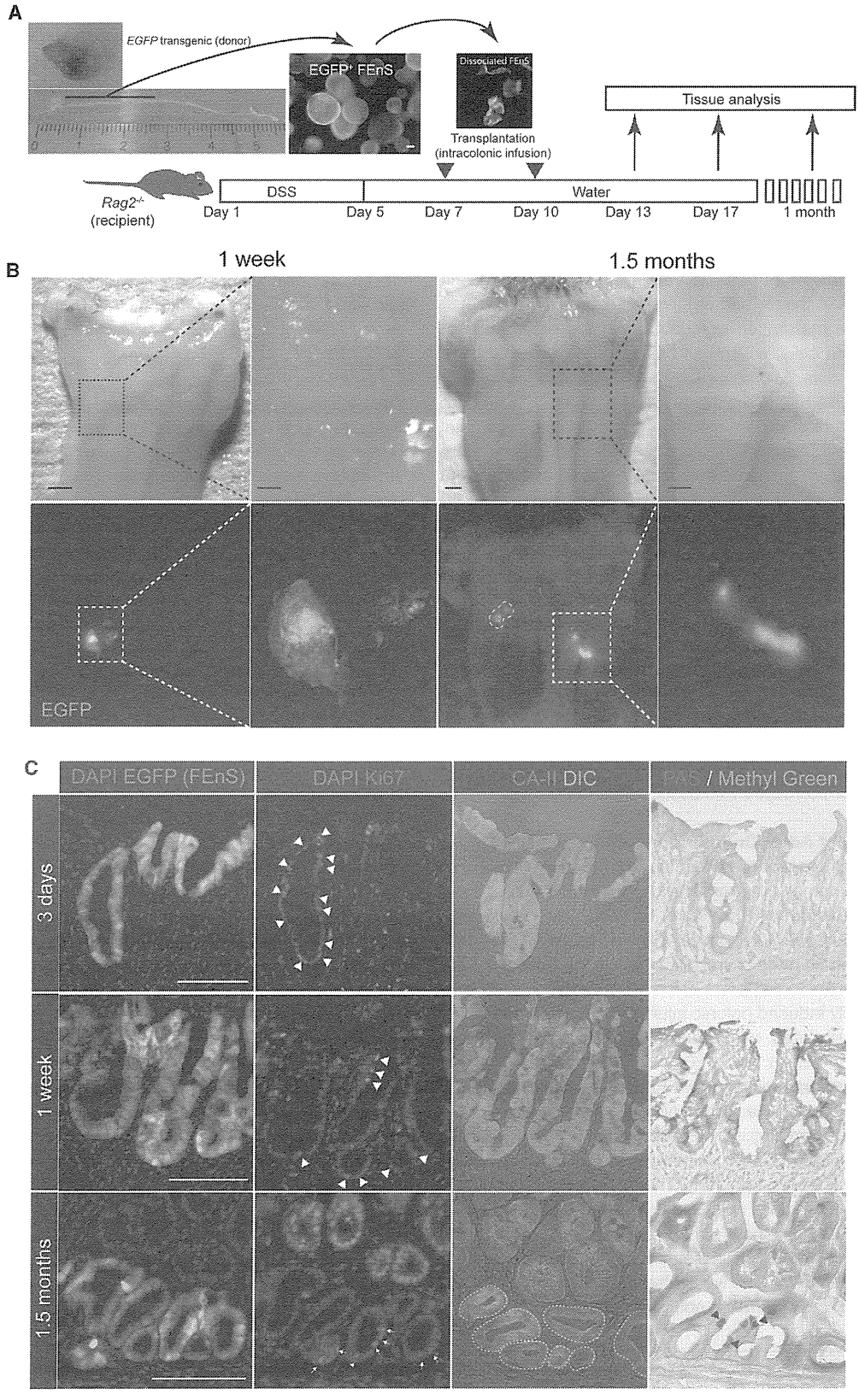

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does remain a possibility that the culture conditions that maintain adult stem cells in vitro are optimal for the distal intestine at this developmental time point rather than a reflection of tissue maturation.

The spatial differences in expression of the Wnt target genes *Lgr5* and *Axin2* (Barker et al., 2007; Lustig et al., 2002) prompted the investigation of Wnt signaling in the developmental transition. The differing requirements between the mature and immature states imply that Wnt signaling has a context-dependent role in development and tissue homeostasis or alternatively that ligands are dynamically regulated. There are several potential Wnt ligands in the intestine, where Wnt3a has been shown to play an autonomous role in epithelial stem cell maintenance (Sato et al., 2011b; Farin et al., 2012). In line with this, we observe that the expression of Wnt3a is correlated with the appearance of adult stem cell markers as well as adult stem cell behavior in the developing epithelium. Interestingly, this pattern of expression coincides with the phenotype of the knockout of the major β-catenin effector, Tcf4, which die shortly after birth with intestinal hypoplasia (Korinek et al., 1998).

In vitro Wnt stimulation and spontaneous maturation can be blocked by Wnt inhibition. Here, Wnt causes a prominent focal upregulation of Lgr5 expression in the developing structures. This is associated with the transition from a thin epithelium to domains with columnar morphology reminiscent of the cellular architecture in the small intestine. After the emergence of Paneth cells, these structures become independent of exogenous Wnt similar to adult intestinal stem cells. We hypothesize that Lgr5 in this context facilitates the transition by enhancing focal Wnt stimulation via the Wnt agonist R-spondin1 (de Lau et al., 2011). This will also explain why established FEnS are resilient to Wnt stimulation in vitro—they express significantly reduced levels of Lgr5. Although Wnt signaling mediates the transitioning of murine FEnS, it might be more complicated for hFEnS, where a low level of Wnt stimulation is required for their normal maintenance.

The gold standard for testing the true differentiation potential of progenitor cells is in vivo transplantation (Lin et al., 2013). We have previously demonstrated that adult colonic organoids can engraft into an injury model (Yui et al., 2012). An initial concern was that due to the striking morphological and growth similarities between FEnS and APC null adult organoids (Sato et al., 2011b), transplantation of FEnS in vivo would lead to tumor formation. However, FEnS cells were shown to attach to denuded regions of colonic epithelium and subsequently be incorporated into the colonic epithelium. Furthermore, since FEnS were unable to survive under the kidney capsule, this suggests that orthotopic transplantation is a more useful readout of in vivo potential. Our transplantation experiments unequivocally demonstrate that established FEnS can mature in vivo and contribute to regeneration of damaged gut epithelium in adult hosts. Moreover, it is striking that these fetal derivatives from the small intestine rapidly respond to the new microenvironment and differentiate appropriately to the regional requirements. This might reflect their immature behavior although we cannot exclude the possibility that adult organoids will behave similarly.

In summary, we have identified a population of expandable fetal enteric progenitors from mouse and human that can be used as a transplantable source. This work has important implications for understanding the mechanisms underlying intestinal maturation and demonstrates that immature intestinal progenitors, including fetal-like material derived from human pluripotent stem cells, have the potential to be used in colonic regenerative medicine. It will be interesting to see if similar populations of immature progenitors exist in other endodermal organs.

EXPERIMENTAL PROCEDURES

Mice

Rag2$^{-/-}$ mice were from Taconic Farms and Central Laboratories for Experimental Animals. *EGFP* transgenic mice and Lgr5-EGFP-ires-CreERT2 mice are described elsewhere (Barker et al., 2007; Okabe et al., 1997). Experimental animals were obtained by crossing these with C57BL/6 male or female animals. All animal experiments in Cambridge were performed under the terms of a UK Home Office License and transplantation experiments were performed with the approval of the Institutional Animal Care and Use committee of TMDU.

Transplantation

Transplantation was performed as described on days 7 and 10 following initiation of dextran sulfate sodium-induced colonic injury (Yui et al., 2012). Donor FEnS were released from the Matrigel and mechanically dissociated into small sheets of epithelial tissue. Cell fragments from 500–1,000 FEnS were resuspended in 200 μl of Matrigel in PBS (1:20), which was instilled into the colonic lumen using a syringe and a thin flexible catheter. Animals were subsequently sacrificed at indicated time points.

In Vitro Cultures

Organoids

Primary crypts from proximal adult small intestine were cultured as previously described with reduced concentration of murine recombinant R-spondin1 (500 ng/ml, R&D Systems; Sato et al., 2009).

FEnS

Fetal small intestines were opened longitudinally and cut into small pieces prior to dissociation with 2 mM EDTA. Isolated epithelial units were embedded in Matrigel and maintained in conditions identical to those used for adult organoids. In certain experiments Wnt3a and R-spondin1 from conditioned media were collected from HEK293 cell lines expressing recombinant Wnt3a and R-spondin1 (kindly provided by Hans Clevers and Calvin Kuo, respectively). Relative Wnt/R-spondin1 activity was measured using a TOPflash assay with a Dual-Luciferase Reporter Assay System (Millipore).

Human Tissue

First-trimester human fetal material was obtained from the John van Geest Centre for Brain Repair, University of Cambridge, and used with informed consent under an Approved Protocol of Human Tissue Studies. Fetuses were staged by Crown Rump Length. Fetal intestines were processed for in vitro epithelial culture, paraffin sections, or RNA extraction, using procedures

Figure 5. Regeneration of Adult Colonic Epithelium from mFEnS

(A) Experimental protocol: gastrointestinal tract dissected from E16 EGFP transgenic mouse fetus (top left). Proximal small intestine was cultured in vitro as FEnS before mechanical dissociation and intracolonic transplantation into *Rag2*$^{-/-}$ adult recipients with Dextran Sulfate Sodium (DSS)-induced ulcerative colitis.

(B) Recipient colon at 1 week and 1.5 months posttransplantation. Lower panel shows EGFP^{+ve} areas in host colon.

(C) Immunohistological analysis of EGFP^{+ve} fetal-derived engraftments for Ki67 (Ki67^{+ve} cells marked by arrowheads), carbonic anhydrase II, and PAS, 3 days, 1 week, and 1.5 months after transplantation.

The scale bars represent 1 mm (whole colons) and 200 μm (magnified areas) in (B) and 100 μm in (C). See also Figure S5.

identical to those described for murine material, with the addition of PGE2 (2.5 μM, Sigma-Aldrich). Adult human intestinal biopsies were obtained from the Division of Gastroenterology and Hepatology, Department of Medicine, University of Cambridge, and were used with local ethical permission, under informed consent.

Adult primary human organoids were derived from biopsies obtained during routine colonoscopies from the terminal ileum. A single crypt suspension was obtained through chelation of the washed biopsies in cold chelation buffer (distilled water with 5.6 mmol/l Na_2HPO_4, 8.0 mmol/l KH_2PO_4, 96.2 mmol/l NaCl, 1.6 mmol/l KCl, 43.4 mmol/l sucrose, 54.9 mmol/l D-sorbitol, 0.5 mmol/l DL-dithiothreitol) containing 4 mM EDTA for 45 min followed by release of the crypts in fresh chelation buffer by vigorous shaking. Isolated crypts were treated like murine and fetal tissues; however, cultures were additionally supplemented with 1xN2 and 1xB27 (from invitrogen), 2.5 mM *N*-acetylcysteine (Sigma), 40% Wnt3a conditioned medium, 10% R-spondin1 conditioned medium, 10 mM nicotinamide, 10 μM SB202190, and 500 nM A-83-01. Tissue for primary human cultures was obtained at Herlev Hospital with local ethical permission and under informed consent.

Generation, Culture, and Differentiation of hiPSCs

hiPSCs (BBHX8) were derived using retrovirus-mediated reprogramming of human skin fibroblasts (Rashid et al., 2010). hiPSCs were cultured in a chemically defined, feeder-free culture system (Brown et al., 2011). Cells were passaged every 7 days using a mixture of collagenase IV or collagenase and dispase at a ratio of 1:1. hiPSCs were differentiated as outlined in Figure S1 and Table S1 (Hannan et al., 2013). Briefly, iPSCs were differentiated into DE using Activin-A, BMP4, and LY294002 for 3 days. DE cells were subsequently cultured with CHIR99021 for 4 days to generate posterior endoderm. Raised aggregates of posteriorized endoderm were transferred into growth factor-reduced Matrigel. The cell-Matrigel mix was overlaid with Advanced DMEM/F12 supplemented with 2 mM GlutaMax (Invitrogen), 10 mM HEPES, and 100 U/ml Penicillin/100 mg/ml Streptomycin containing B27 supplement, Y-27632 (10 mM), human Noggin (100 ng/ml), human EGF (100 ng/ml), human R-spondin1 (1 mg/ml), and human Wnt3a (100 ng/ml).

Imaging and Histology

Live imaging of 3D cultures was performed using a Nikon Biostation IM system. Structures in Matrigel were observed using phase contrast and DIC microscopy using an Axiovert 200M microscope (Zeiss) equipped with an AxioCam MRc (Zeiss).

Tissue preparation, staining, and image analysis were carried out as described previously using antibodies listed in Table S2 (Wong et al., 2012; Yui et al., 2012). Images of sections were acquired using a DeltaVision system (Applied Precision) or a Zeiss Imager M.2, equipped with AxioCam MRm and MRc cameras.

DIG in situ hybridization was carried out essentially as described before using IMAGE clones (Gregorieff et al., 2005).

RNA Extraction and qRT-PCR

RNA was isolated from intact intestine as described (Wong et al., 2012). Total RNA was isolated from cultured cells using the Invitrogen PureLink RNA micro kit. cDNA was synthesized from 100 ng total RNA using the Invitrogen SuperScript III Reverse transcriptase kit, using random primers. Gene-specific expression assays (Applied Biosystems) or SYBR Green analysis (Invitrogen) with optimized primer pairs was used for qPCR on an Applied Biosystems 7500HT RealTime PCR System (Applied Biosystems). Values were normalized to 18S using the ΔCt method. Z scores were calculated and used to generate heatmaps in R.

Isolation of Cells for Flow Cytometry

Cells were isolated essentially as described (Wong et al., 2012). A single-cell suspension was achieved by subsequent incubation using trypsin. Cell sorting was carried out using a MoFlo (Beckman Coulter). Ten thousand cells were seeded into 25 μl Matrigel. Data analysis was performed in FlowJo.

Statistical Analysis

Statistical significance of quantitative data was determined by applying a two-tailed Student's t test to raw values or to the average values obtained from analysis of independent experiments. A two-tailed Fisher's exact test was used to analyze the significance of the Wnt and inhibitor culture experiment.

SUPPLEMENTAL INFORMATION

Supplemental Information for this article includes five figures, two tables, and two movies and can be found with this article online at http://dx.doi.org/10.1016/j.stem.2013.09.015.

AUTHOR CONTRIBUTIONS

R.P.F., S.Y., and K.B.J. conceived and designed the study, analyzed the data, and wrote the manuscript; R.P.F., S.Y., N.R.F.H., C.S., A.M., P.J.S., and K.B.J performed experimental work; and R.P.F., S.Y., and K.B.J. prepared the figures. O.H.N., L.V., R.A.P., and M.W. gave conceptual advice. T.N. and K.B.J supervised the project.

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A Genome-Wide Association Study Identifies 2 Susceptibility Loci for Crohn's Disease in a Japanese Population

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BACKGROUND & AIMS: Crohn's disease is an inflammatory bowel disease induced by multiple genetic and environmental factors. Genome-wide association studies have identified genetic factors that affect the risk for Crohn's disease in European populations, but information from other ethnic groups is scarce. We therefore investigated genetic factors associated with Crohn's disease in the Japanese population. **METHODS:** We performed a genome-wide association study with 372 individuals with Crohn's disease (cases) and 3389 controls, all from the Japanese population. To confirm identified associations, we performed a replication study with an independent panel of 1151 Crohn's disease cases and 15,800 controls. We also performed an association analysis using genome-wide genotype imputation in the discovery cohort. **RESULTS:** We confirmed associations of Crohn's disease with variants in *MHC* (rs7765379, $P = 2.11 \times 10^{-59}$), *TNFSF15* (rs6478106, $P = 3.87 \times 10^{-45}$), and *STAT3* (rs9891119, $P = 2.24 \times 10^{-14}$). We identified 2 new susceptibility loci: on chromosome 4p14 (rs1487630, $P = 2.40 \times 10^{-11}$; odds ratio, 1.33), and in the *SLC25A15–ELF1-WBP4* region on 13q14 (rs7329174 in ELF1, $P = 5.12 \times 10^{-9}$; odds ratio, 1.27). **CONCLUSIONS: In a genome-wide association study, we identified 2 new susceptibility loci for Crohn's disease in a Japanese population. These findings could increase our understanding of the pathogenesis of Crohn's disease.**

Keywords: Inflammatory Bowel Disease; GWAS; Polymorphism; CD.

Crohn's disease (CD) (Online Mendelian Inheritance in Man [OMIM] MIM 266600; available at http://www.ncbi.nlm.nih.gov/omim?db=omim), one of the main forms of inflammatory bowel disease (IBD), is a multifactorial disease in which many genetic and environmental factors contribute to its development.[1] Although the prevalence of CD is lower (23.6/100,000 persons) in Japan compared with European populations,[2] it has been increasing continuously over the past several decades in Japan and other Asian countries.[3] Evidence suggests that abnormal responses of the mucosal immune system to intestinal microbes in a genetically susceptible host play a major role in the pathogenesis of CD.[4,5]

To clarify the pathogenesis of CD, genome-wide association studies (GWASs) since 2005 have identified various susceptibility loci, including tumor necrosis factor (ligand) superfamily, member 15 (*TNFSF15*),[6] interleukin 23 receptor (*IL23R*),[7] autophagy related 16-like 1 (*ATG16L1*),[8,9] the gene desert region on chromosome 5p13.1,[10] and immunity-related GTPase family, M (*IRGM*).[11–13] In 2010, a meta-analysis of 6 GWAS reported 71 susceptibility loci to CD in the European population, but those loci were estimated to explain only 23.2% of heritability.[14] That result indicates that a large number of CD susceptibility loci have not yet been identified.[15] Although there are no significant differences in the clinical characteristics and natural history of CD between Asians and Europeans,[16,17] studies have shown apparent ethnic differences between European and East-Asian populations for CD susceptibility loci such as nucleotide-binding oligomerization domain containing 2 (*NOD2*), *IL23R*, and *ATG16L1*.[18–20]

To clarify the genetic background of IBD pathology in the Japanese population, we previously analyzed nearly 80,000 gene-based markers and identified *TNFSF15* as a susceptibility gene in the Japanese population.[6] By comparative GWAS of 2 IBD subtypes using the Illumina HumanHap550v3 chip (Illumina, San Diego, CA), we later showed that HLA-Cw*1202-B*5201-DRB1*1502 in-

Abbreviations used in this paper: CD, Crohn's disease; CI, confidence interval; ELF1, erythroid-like transcription factor family 1; eQTL, estimated cis–expression quantitative trait loci; GRAIL, Gene Relationships Across Implicated Loci; GWAS, genome-wide association study; HWE, Hardy–Weinberg equilibrium; IBD, inflammatory bowel disease; LD, linkage disequilibrium; MAF, minor allele frequency; MHC, major histocompatibility complex; OR, odds ratio; PCA, principal component analysis; PCR, polymerase chain reaction; SNP, single nucleotide polymorphisms; TLR, Toll-like receptor.

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Table 1. Basic Characteristics of Study Subjects

	Case	Control
GWAS		
Platform	Illumina HumanHap550	Illumina HumanHap550
Number of samples	372	3389
Ethnicity	Japanese	Japanese
Male (%)	266 (71.5)	1846 (54.5)
Age at sampling, y[a]	33.8 ± 9.3	52.5 ± 15.2
Replication		
Platform	Multiplex PCR ad Invader assay	Illumina HumanHap550 Illumina Human Hap610-Quad
Number of samples	1151	15,800
Ethnicity	Japanese	Japanese
Male (%)	789 (68.6)	10,427 (66.0)
Age at sampling, y[a]	34.4 ± 13.9	63.7 ± 10.5

[a]Mean ± standard deviation.

creases the risk for ulcerative colitis (MIM 191390) but confers a protective effect for CD.[21] Here, we report a GWAS using 372 individuals with CD and 3389 controls with a replication analysis of the top associations in an independent panel of 1151 Crohn's disease cases and 15,800 controls.

Patients and Methods

Subjects Enrolled in the Study

A total of 1535 individuals with CD and 19,283 control subjects were enrolled in this study. All individuals were of Japanese descent. Characteristics of the study subjects are shown in Table 1. CD cases for the GWAS were collected at the Social Insurance Chuo General Hospital (n = 376; same set as the previous study[21]). Cases for the replication study were a total of 1159 subjects collected at the Social Insurance Chuo General Hospital (n = 160), Toho University Sakura Medical Centre (n = 41), Sapporo Medical University School of Medicine and Sapporo Kosei Hospital (n = 351), and Kyushu University and 16 affiliated hospitals (n = 607). All CD cases were diagnosed by expert gastroenterologists in accordance with clinical, radiologic, endoscopic, and histologic features according to the Lennard-Jones[22] criteria. Patients with indeterminate colitis were excluded. The control subjects for the GWAS consisted of 3397 subjects from 2 groups, healthy volunteers (n = 906) recruited at the Midosuji and other related Rotary Clubs,[6,20,21,23] and subjects (n = 2491) obtained from the BioBank Japan project (http://biobankjp.org/) who had been enrolled based on having one of various disease classes (cervical cancer, esophageal cancer, gallbladder cancer, liver cancer, ovarian cancer, pancreatic cancer, hematologic cancer, endometrial cancer, chronic hepatitis B, keloid, drug eruption, febrile seizure, and pulmonary tuberculosis).[24] For replication study controls, we used a nonoverlapping set of samples from the BioBank Japan project that consisted of 15,800 individuals classified into 1 of 10 disease categories (colorectal cancer, breast cancer, prostate cancer, lung cancer, stomach cancer, diabetes, arteriosclerosis obliterans, atrial fibrillation, cerebral infarction, and myocardial infarction). The study was approved by the ethical committees of each participating medical center, the Institute of Medical Science, the University of Tokyo, and the Riken Yokohama Institute.

Single-Nucleotide Polymorphism Genotyping and Quality Controls

In the GWAS, 376 CD cases and 3397 controls were genotyped using the Illumina HumanHap550v3 Genotyping BeadChip (Illumina). We excluded 2 CD cases with call rates lower than 0.98. After searching for close relatives using identity-by-descent estimated by PLINK (available at http://pngu.mgh.harvard.edu/~purcell/plink/), we excluded 2 cases and 8 control subjects. To evaluate potential stratification in our study population, we merged the GWAS data with European (CEU; Utah residents [CEPH] with Northern and Western European ancestry), African (YRI; Yoruba in Ibadan, Nigeria), and East-Asian (Japanese and Han Chinese; JPT; Japanese in Tokyo, Japan and CHB; Han Chinese in Beijing, China) individuals' genotype data obtained from the Phase II HapMap database (release 22)[25] and performed a principal component analysis (PCA). A PCA plot clearly separated the subjects into 3 clusters as previously described (Supplementary Figure 1).[26] We applied stringent quality control criteria to the single-nucleotide polymorphism (SNP) data, with a genotype call rate of 0.99 or greater in both cases and controls, Hardy–Weinberg equilibrium (HWE) $P \geq 1 \times 10^{-6}$ in controls, and minor allele frequency (MAF) greater than 0.01 in both cases and controls. After quality control filtering, a total of 453,099 SNPs consisting of 442,912 autosomal and 10,187 chromosome X SNPs were used for further analysis.

For the replication study, we selected 98 SNPs with $P < 1 \times 10^{-4}$ in the GWAS after excluding SNPs located in previously reported loci in the Japanese population.[6,27] After calculating r^2 among the 98 SNPs we examined groups of SNPs with $r^2 \geq 0.9$ to other candidate SNPs and selected the 65 SNPs that had the lowest P value within each putative locus. Among them, 1 SNP (rs6739399) was excluded because genotype data for controls were not available in the replication set. Another SNP (rs6966614) was excluded because of the difficulty of assay design. A total of 63 SNPs were genotyped using the multiplex polymerase chain reaction (PCR)-based Invader assay (Third Wave Technologies, Madison, WI). We excluded 8 cases with call rates less than 0.98 in the replication study. The concordance rate between genotypes determined by the Illumina HumanHap550v3 BeadChip and those by the Invader assay among 372 duplicated samples in 30 SNPs was 99.90%. The controls for the replication study were genotyped using the Illumina HumanHap610-Quad BeadChip. Genotype concordance between HumanHap550v3 and HumanHap610-Quad BeadChips was 99.99% among 182 duplicate samples, indicating a low possibility of genotype error. As a result, 63 SNPs were analyzed in 1151 cases and 15,800 controls in the replication study.

Genome-Wide Imputation and Replication Analysis

By using IMPUTE2 (available at http://mathgen.stats.ox.ac.uk/impute/impute_v2.html),[28] genome-wide imputation was conducted separately for the 3738 GWAS subjects (372 cases and 3366 controls). The reference haplotypes consisted of data for 286 East-Asian subjects (CHB, 97; CHS, 100; JPT, 89) from the 1000 Genomes Project March 2012 release (phase 1, version 3).[29] The GWAS samples' imputed genotype data had 4,929,034 SNPs with an MAF of more than 5% and an estimated imputation accuracy of greater than 0.9 (IMPUTE2 info).

From analysis of the imputed GWAS data, we found 44 additional candidate lead SNPs representing putative novel susceptibility loci for CD ($P < 1 \times 10^{-4}$). We performed genotyping