# Patients with colorectal cancer have identical strains of *Fusobacterium nucleatum* in their colorectal cancer and oral cavity

We read with great interest the article by Flemer *et al*, which suggests that analysis of the oral microbiota could potentially be used as a screening method for colorectal cancer (CRC) and polyp detection.[1] *Fusobacterium* (*F.*) *nucleatum* is one of the most densely colonised bacterial species in the oral cavity and is known to be associated with periodontitis.[2] Recently, many researchers have demonstrated that *F. nucleatum* is related to CRC development and pathogenicity.[3 4] However, the relationship between *F. nucleatum* in CRC and the oral cavity is not well understood. For this purpose, we examined whether identical strains of *F. nucleatum* could be isolated from CRC and saliva specimens obtained from the same patient. The approach used in this study is detailed in figure 1A (see online supplementary information for details). We collected CRC and saliva samples from 14 patients (online supplementary table 1) and isolated bacteria from the specimens on *Fusobacterium*-selective agar. All colonies (1,351 in total) were analysed by PCR using *F. nucleatum*-specific primer sets, and 361 *F. nucleatum* isolates were obtained. *F. nucleatum* was detected in 8 of 14 patients (57.1%) from CRC samples and in all patients (100%) from saliva samples (figure 1B). The *F. nucleatum* subspecies identified by 16S rRNA gene sequencing and the number of isolates from each specimen are shown in table 1.

Four subspecies, *F. nucleatum* subsp. *animalis*, *F. nucleatum* subsp. *nucleatum*, *F. nucleatum* subsp. *polymorphum* and *F. nucleatum* subsp. *vincentii* were isolated from the samples. To identify *F. nucleatum* isolates from CRC and saliva at the strain level, we performed arbitrarily primed PCR (AP-PCR) as the strain typing method, which can be applied without genome information or

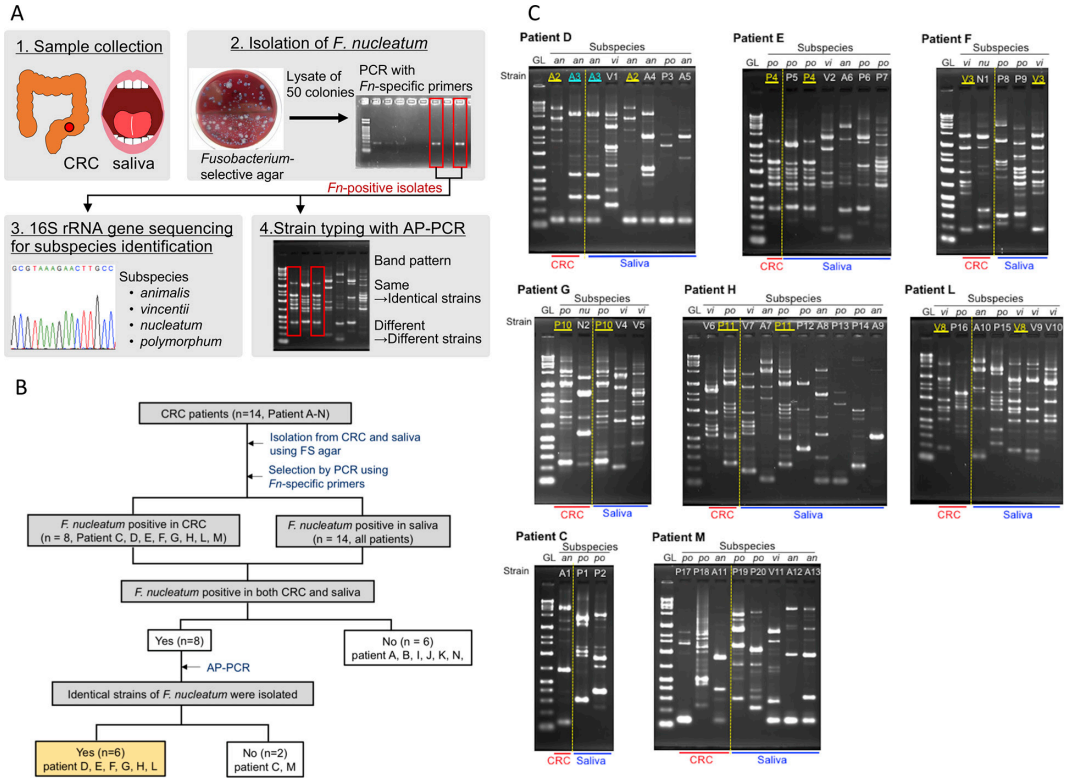


**Figure 1** Detection of *Fusobacterium nucleatum* subspecies in paired colorectal cancer and saliva samples. (A) Schematic of the experimental procedures. AP-PCR, arbitrarily primed PCR; CRC, colorectal cancer; Fn, *Fusobacterium nucleatum*. See online supplementary information for more details. (B) Flowchart of the study process. FS agar, *Fusobacterium*-selective agar. (C) AP-PCR patterns detected with primer D11344. Data are representative of at least two independent experiments. Identical pairs are highlighted in yellow or blue. GL, gene ladder (0.1, 0.2, 0.3, 0.4, 0.5, 0.7, 1.0, 1.3, 1.5, 2.0, 3.0, 4.0, 5.0, 7.0, 10 and 20 kbp). Subspecies, an, nu, po and vi are *F. nucleatum* subsp. *animalis*, *F. nucleatum* subsp. *nucleatum*, *F. nucleatum* subsp. *polymorphum* and *F. nucleatum* subsp. *vincentii,* respectively.

**Table 1** Subspecies and strains detected in each patient

| *Fusobacterium nucleatum* subspecies | Number of isolates | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Patient C | | Patient D | | Patient E | | Patient F | | Patient G | | Patient H | | Patient L | | Patient M | |
| | CRC | Saliva | CRC | Saliva | CRC | Saliva | CRC | Saliva | CRC | Saliva | CRC | Saliva | CRC | Saliva | CRC | Saliva |
| *animalis* | | | | | | | | | | | | | | | | |
| Strain A1 | 21 | 0 | | | | | | | | | | | | | | |
| Strain A2 | | | 13* | 3* | | | | | | | | | | | | |
| Strain A3 | | | 1* | 5* | | | | | | | | | | | | |
| Strain A4 | | | 0 | 1 | | | | | | | | | | | | |
| Strain A5 | | | 0 | 2 | | | | | | | | | | | | |
| Strain A6 | | | | | 0 | 1 | | | | | | | | | | |
| Strain A7 | | | | | | | | | | | 0 | 2 | | | | |
| Strain A8 | | | | | | | | | | | 0 | 1 | | | | |
| Strain A9 | | | | | | | | | | | 0 | 1 | | | | |
| Strain A10 | | | | | | | | | | | | | 0 | 3 | | |
| Strain A11 | | | | | | | | | | | | | | | 1 | 0 |
| Strain A12 | | | | | | | | | | | | | | | 0 | 1 |
| Strain A13 | | | | | | | | | | | | | | | 0 | 1 |
| *nucleatum* | | | | | | | | | | | | | | | | |
| Strain N1 | | | | | | | 11 | 0 | | | | | | | | |
| Strain N2 | | | | | | | | | 1 | 0 | | | | | | |
| *polymorphum* | | | | | | | | | | | | | | | | |
| Strain P1 | 0 | 1 | | | | | | | | | | | | | | |
| Strain P2 | 0 | 1 | | | | | | | | | | | | | | |
| Strain P3 | | | 0 | 1 | | | | | | | | | | | | |
| Strain P4 | | | | | 1* | 5* | | | | | | | | | | |
| Strain P5 | | | | | 0 | 3 | | | | | | | | | | |
| Strain P6 | | | | | 0 | 1 | | | | | | | | | | |
| Strain P7 | | | | | 0 | 1 | | | | | | | | | | |
| Strain P8 | | | | | | | 0 | 3 | | | | | | | | |
| Strain P9 | | | | | | | 0 | 1 | | | | | | | | |
| Strain P10 | | | | | | | | | 47* | 3* | | | | | | |
| Strain P11 | | | | | | | | | | | 1* | 3* | | | | |
| Strain P12 | | | | | | | | | | | 0 | 5 | | | | |
| Strain P13 | | | | | | | | | | | 0 | 1 | | | | |
| Strain P14 | | | | | | | | | | | 0 | 2 | | | | |
| Strain P15 | | | | | | | | | | | | | 0 | 7 | | |
| Strain P16 | | | | | | | | | | | | | 1 | 0 | | |
| Strain P17 | | | | | | | | | | | | | | | 43 | 0 |
| Strain P18 | | | | | | | | | | | | | | | 3 | 0 |
| Strain P19 | | | | | | | | | | | | | | | 0 | 26 |
| Strain P20 | | | | | | | | | | | | | | | 0 | 8 |
| Strain P21 | | | | | | | | | | | | | | | 0 | 1 |
| *vincentii* | | | | | | | | | | | | | | | | |
| Strain V1 | | | 0 | 3 | | | | | | | | | | | | |
| Strain V2 | | | | | 0 | 2 | | | | | | | | | | |
| Strain V3 | | | | | | | 2* | 2* | | | | | | | | |
| Strain V4 | | | | | | | | | 0 | 3 | | | | | | |
| Strain V5 | | | | | | | | | 0 | 1 | | | | | | |
| Strain V6 | | | | | | | | | | | 48 | 0 | | | | |
| Strain V7 | | | | | | | | | | | 0 | 21 | | | | |
| Strain V8 | | | | | | | | | | | | | 32* | 1* | | |
| Strain V9 | | | | | | | | | | | | | 0 | 1 | | |
| Strain V10 | | | | | | | | | | | | | 0 | 1 | | |
| Strain V11 | | | | | | | | | | | | | | | 0 | 6 |

Strain P21 did not grow from stock.
*Strains detected from both specimens.

specialised techniques and equipment.[5–7] We performed AP-PCR targeting the *F. nucleatum* isolates from the 8 patients whose CRC and saliva samples were both *F. nucleatum*-positive and analysed the detected AP-PCR patterns (figure 1C and online supplementary figure 1). Focusing on patient C (left, bottom), there were no common isolates between their CRC and saliva samples (figure 1C). However, patient D (left, top) had two and four strains of *F. nucleatum* subsp. *animalis* detected in their CRC and saliva, respectively. Furthermore, strains A2 and A3 (highlighted in yellow and blue) were indicated as identical strains by the AP-PCR patterns (figure 1C). We detected identical *F. nucleatum* strains in both CRC and saliva from 42.9% (6/14) of the patients. Notably, an identical strain was detected in 75% (6/8) of patients who were both *F. nucleatum*-positive in CRC and saliva specimens. From our results, there were no significant differences in the detection rate of *F. nucleatum* among each lesion site from the 8 patients. *F. nucleatum* was detected from stages 0 to IV (online supplementary table 1), indicating that *F. nucleatum* could adhere to CRC tissue from an early stage of tumorigenesis, as previously reported.[8 9] From our results,more than 40% of CRC patients exhibited identical strains of *F. nucleatum* in their CRC and saliva specimens. This suggests that *F. nucleatum* in CRC originates in the oral cavity. Our findings support that targeting *F. nucleatum* in the oral cavity may provide insights for further studies in the field of human microbiome research and CRC.

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