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## INTRODUCTION

The presence of an oral commensal, *Fusobacterium nucleatum*, in colorectal cancer (CRC) has been identified as an indicator of poor prognosis and has also been shown to increase gradually from stage I to IV. Several adhesion molecules in *F. nucleatum*, including RadD, FadA and Fap2, have been identified as virulence factors in CRC. However, given that *F. nucleatum* contains over 2,000 genes, it is possible that additional undiscovered pathogenic factors are contributing to *F. nucleatum*-induced CRC stimulation.

## OBJECTIVES

- (1) Identification of potential pathogenic genes involved in CRC progression;
- (2) Evaluation of biofilm properties of clinical *F. nucleatum* isolates and their carcinogenicity in CRC cell line.

## MATERIALS

- F. nucleatum* wild types (23726 and 25586) were provided by Dr. Chenggang Wu from the University of Texas Health Science Center in Houston.
- 19 of clinical *F. nucleatum* were collected from healthy and OSCC patients' saliva in Taiwan.

	OSCC	Non-OSCC
Patients	101	158
Viable <i>Fusobacterium</i> sp.	11	15
Isolation rate (%)	10.89%	9.49%

	OSCC	Non-OSCC
<i>F. nucleatum</i> subsp. <i>polymorphum</i>	24438, 24915, 26503, 30775, 31431, 31576, 31742, 33399, 33804	25210, 25749, 26560, 30065, 31000, 33357
<i>F. nucleatum</i> subsp. <i>animalis</i>	29703	26998, 30979
<i>F. nucleatum</i> subsp. <i>vincentii</i>	-	25544
<i>F. periodonticum</i>	31762	24639, 25525, 25712, 30795, 31020, 33060

**Table 1. Information for clinical *F. nucleatum* isolated from Taiwanese patients' saliva.** A total of 10 *F. nucleatum* isolated from OSCC patients and 9 from non-OSCC patients.

- Human colorectal cancer cell line (HCT116) was purchased from the American Type Culture Collection (ATCC).

## ACKNOWLEDGEMENTS

### Funding sources

- OSU-CHS startup (Dr. I-Hsiu Huang)
- Cancer Sucks Inc., Bixby, Oklahoma (Dr. Rashmi Kaul)

### Collaborators

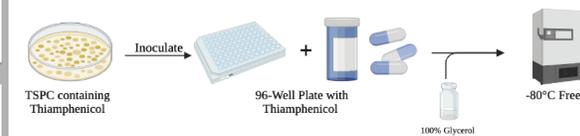
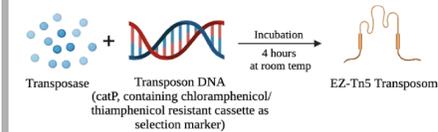
- Dr. Chenggang Wu (UTHSC-Houston)
- Dr. Hung Ton-That (UCLA)
- Dr. Jenn-Wei Chen (National Cheng Kung University)
- Dr. Subhas Das (OSU-CHS)
- Dr. Rashmi Kaul (OSU-CHS)

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- Chenggang W, et al. (2018) Forward genetic dissection of biofilm development by *Fusobacterium nucleatum*: novel functions of cell division proteins FtsX and EnvC. *mBio* 9:e00360-18.
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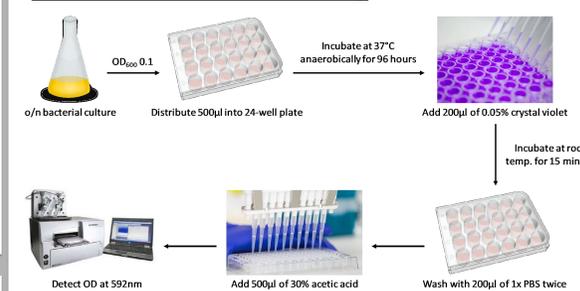
## METHODS

### (1) EZ-Tn5 Transposon mutant library

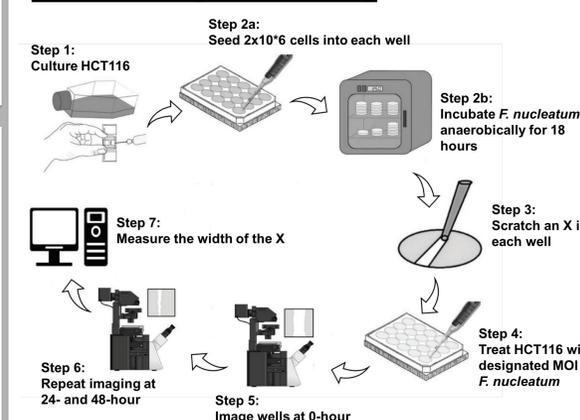


### (2) Assays on *F. nucleatum* clinical isolates

#### - Biofilm formation assay



#### - CRC Cell migration assay



## RESULTS and CONCLUSIONS

### (1) EZ-Tn5 Transposon mutant library

A transposon mutant library of 9,600 mutant colonies of *F. nucleatum* was created, with a minimum genome coverage of 99% achieved.

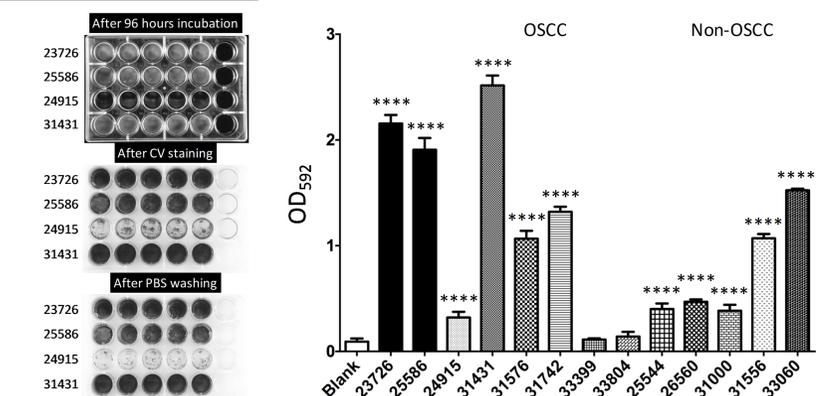
P=1-(1-f)^N		
P	f	N
0.991621	(2,299,539/2,008)/2,299,539=0.0004980079681	9,600

**Table 2. Calculation for mutant library coverage.** N: colony number; P: coverage (%); f: gene length/whole genome.

### (2) Assays on *F. nucleatum* clinical isolates

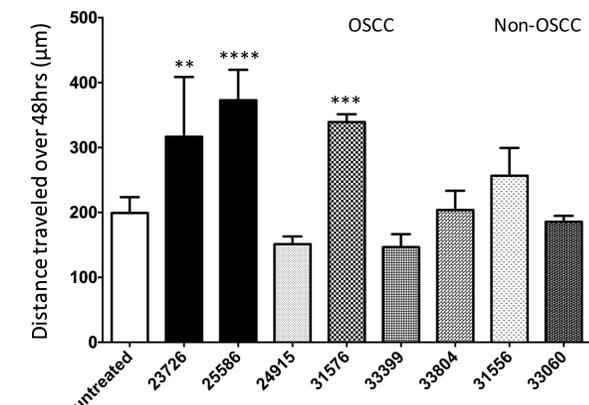
Clinical isolates from both OSCC and non-OSCC patients demonstrated varying levels of biofilm formation ability and stability, as well as CRC cell migration.

#### - Biofilm formation assay



**Figure 1. Statistical Analysis of Biofilm Formation.** Measure of bacterial biofilm after 96 hours of incubation.

#### - CRC cell migration assay



**Figure 2. Statistical Analysis of Cell Migration.** The distance traveled was measured at 48 hours after HCT116 treated with fresh medium, and MOI 10 of wild type 23726 and 25586, Taiwanese OSCC isolates 24915, 31576, 33399, 33804 and non-OSCC isolates 31556 and 33060.