BACTERIOPHAGE DELIVERY INTO TUMOR ASSOCIATED BACTERIA

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Introduction

Recent studies revealed that bacterial species present within the tumor microenvironment (Bhatt et al., 2017) may promote tumor growth (Rubinstein et al., 2013 & Yang et al., 2017), enable tumor cells to evade the immune system (Gur et al., 2015) and provide resistance to cancer therapeutics (Yu et al., 2017 & Geller 2017). *Fusobacterium nucleatum* (*F. nucleatum*) was shown to be significantly enriched in human colorectal carcinomas, pancreatic, breast, esophageal and stomach cancer and adenocarcinomas (Abed et al., 2017), compared with matched adjacent noncancerous tissue or tissue from healthy controls. A higher abundance of *F. nucleatum* within tumors has been associated with advanced tumor stage and poor prognosis in patients with colorectal carcinomas (Mima et al., 2016). Bacteriophage ('phage') are viruses that specifically infect bacteria, and as such play a critical role in regulating bacterial populations. Thus phage may serve as an anti-bacterial candidate therapy for tumor-associated bacteria. The present study aimed to demonstrate phage delivery into tumor associated *F. nucleatum* bacteria.

Methodology

- **Quantitative polymerase chain reaction (qPCR) was applied to detect F. nucleatum in CRC and normal tissues.**
- **Natural phages were isolated from clinical samples, sequenced and tested against *F. nucleatum* strains.**
- **To study *in vivo* phage delivery, a syngeneic subcutaneous mouse model of murine colon carcinoma (CT26) was used. *F. nucleatum* was injected IV and presence in the tumor environment was determined by extraction and colony counts (CFU). Phage delivery was carried out by a single IV administration and phage abundance in serum and in tumors was determined by counting plaque forming units (PFU) on lawns of *F. nucleatum*.**
- **Phage infection of intra-tumoral *F. nucleatum* was measured by a qPCR designed to specifically detect *F. nucleatum* phage DNA incorporated in the host bacterial DNA.**

Results

1. High prevalence of *F. nucleatum* in colorectal cancer tumors

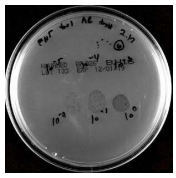
Research group (publication date)	Total cases (n)	% positive	Detection method	CRC Sample type
Li et al (2016)	101	87%	qPCR	Frozen tissue
BiomX	60	84%	qPCR	Frozen tissue

Probe-based qPCR was performed to determine *F. nucleatum* levels in colorectal cancer tissues. Levels of the *F. nucleatum* specific NusG gene (encoding a transcription antitermination protein unique to *F. nucleatum)* and of a human specific internal control gene (PGT) were measured simultaneously on DNA isolated with the Qiamp DNA Mini Kit (QIAGEN, Hilden, Germany). Samples were scored positive for *F.nucleatum* if $2^{-\Delta CT}$ was higher than 0.0002

2. Novel Phages for *F. nucleatum*

More than 10 novel natural phages for *F. nucleatum* were discovered and characterized for host range on clinical *F. nucleatum* isolates. Both lysogenic and lytic phages recognizing the different *F. nucleatum* subspecies (*animalis, vincentii, nucleatum and polymorphum*) were isolated. Two highly novel dsRNA phage were identified.

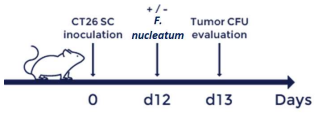
Cleared lytic zones (plaques) of phages specific for *F. nucleatum* strains.



3. *F. nucleatum* homes to CRC tumors *in vivo*

A representative *F. nucleatum* strain was used to colonize tumors in a syngeneic mouse subcutaneous model. Following 12 days of tumor growth, bacteria was administered by IV, and specific CFU content of the tumor was determined 24h later. The study included 4 groups of n=10 mice each as shown below.

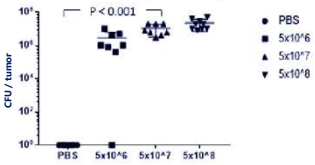
Study Design



Group	Mice per group (n)	CT26 # per subcutaneous injection	*F. nucleatum* # per Injection
1	10	$5x10^5$ cells/mouse	PBS
2	10		$5x10^6$ *Fn*/mouse
3	10		$5x10^7$ *Fn*/mouse
4	10		$5x10^8$ *Fn*/mouse

Results

Total CFU/tumor (mean +/- SD)

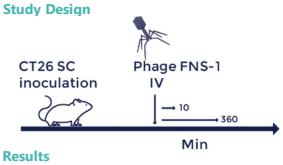


Following IV administration, *F. nucleatum* accumulated within the tumors (10^6-10^8 CFU/gr tumor) in a dose dependent manner.

4. Pharmacokinetics of phages following IV administration to tumor bearing mice

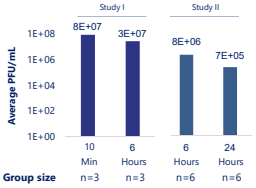
Tumor bearing mice were administrated 10^9 pfu/mL IV of a selected phage, and phage levels were evaluated at several time points in both tumors and serum.

Study Design

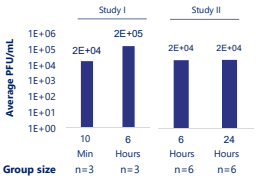


Results

Serum



Tumor

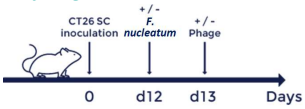


Following IV administration of $2*10^8$ pfu/mouse, phage levels in serum were ~10^7 PFU/mL for at least 6 hours and were reduced after 24 hours to ~10^5 PFU/mL. In the tumor levels remain stable for 24 hours (10^4-10^5). We conclude that phage levels in the tumor are stable for at least several hours and can be used in future applications.

5. Phage delivery to intra-tumor *F. nucleatum*

To study if phages can reach and infect *F. nucleatum* that is embedded within the tumor microenvironment, we employed lysogenic phage whose incorporation into the bacterial DNA could be followed by qPCR. On day 13, 24h following *F. nucleatum* administration to tumor bearing mice, phage were administered by IV. The presence of lysogenic F.nucleatum phage DNA embedded in intratumor *F. nucleatum* was detected as the difference in CT between the reaction measuring Lysogenic phage and that measuring total *F. nucleatum* bacteria (NusG).

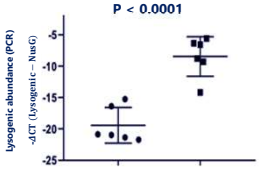
Study Design



Results

Mean qPCR of lysogenic +/- SD

P < 0.0001



IV adm. *F. nucleatum*	+	+
IV adm. Phage	-	+

The abundance of the *F. nucleatum* lysogenic phage genome incorporated in the bacterial DNA was analyzed after normalizing to the total *F. nucleatum* content, that was determined by NusG quantitation using qPCR. Statistical analyses were performed using a two-tailed t-test. A statistically significant increase (P<0.0001) in temperate phage incorporation onto *F. nucleatum* was observed in tumors derived from mice to which both *F. nucleatum* and phage had been administered compared to mice that received *F. nucleatum* only.

Discussion and Conclusions

- Eradication of intra-tumoral *F. nucleatum* may be a potential new modality for treatment of cancer given its high prevalence and abundance within colonic tumors and correlation of the presence of this bacteria with poor prognosis (Mima et al., 2016, Flanagan et al., 2014).
- IV administered *F. nucleatum*-specific phage were able to reach tumors and target intra-tumoral bacteria, thus providing a potential novel therapeutic approach to achieve eradication of these pathogenic tumor associated bacteria.
- We further plan to develop payload bearing phage, which can be used to deliver an anti-cancer payload into the tumor and may act independently or in conjunction with current anti-tumor therapies.

References

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