Sticking to the Problem: Engineering Adhesion in Molecular Endoscopic Imaging

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Abstract—Cancers of the digestive tract cause nearly one quarter of the cancer deaths worldwide, and nearly half of these are due to cancers of the esophagus and colon. Early detection of cancer significantly increases the rate of survival, and thus it is critical that cancer within these organs is detected early. In this regard, endoscopy is routinely used to screen for transforming/cancerous (i.e. dysplastic to fully cancerous) tissue. Numerous studies have revealed that the biochemistry of the luminal surface of such tissue within the colon and esophagus becomes altered throughout disease progression. Molecular endoscopic imaging (MEI), an emerging technology, seeks to exploit these changes for the early detection of cancer. The general approach for MEI is as follows: the luminal surface of an organ is exposed to molecular ligands, or particulate probes bearing a ligand, cognate to biochemistry unique to pre-cancerous/cancerous tissue. After a wash, the tissue is imaged to determine the presence of the probes. Detection of the probes post-washing suggests pathologic tissue. In the current review we provide a succinct, but extensive, review of ligands and target moieties that could be, or are currently being investigated, as possible cognate chemistries for MEI. This is followed by a review of the biophysics that determines, in large part, the success of a particular MEI design. The work draws an analogy between MEI and the well-advanced field of cell adhesion and provides a road map for engineering MEI to achieve assays that yield highly selective recognition of transforming/cancerous tissue *in situ*.

Keywords—Endoscopy, Cell adhesion, Cancer, Dysplasia, Esophagus, Colon, Gastrointestinal.

INTRODUCTION

Nearly half of the cancers of the gastrointestinal (GI) tract occur in the esophagus and colon.^{6,74} There is a critical need for assays that enable the early detection of cancer within these organs and endoscopy is often used for this purpose. Numerous studies have revealed that the luminal surface of the colon/esophagus is biochemically altered throughout disease progression. Molecular endoscopic imaging (MEI), an emerging technology, seeks to exploit these changes for the early detection of cancer. In this review we: (i) provide a succinct but extensive review of biochemical changes that could be, or are currently being, exploited for MEI; (ii) review the biophysics that determines, in large part, the success of a particular MEI design; and (iii) outline an engineering approach for developing MEI assays *in situ*.

BIOCHEMICAL ALTERATIONS DURING ESOPHAGEAL AND COLONIC ONCOGENESIS

The biochemical changes that are present at the apical, i.e. luminal surface, of the esophagus and the colon are primarily relevant to MEI. Although intracellular changes and changes in tissue layers beneath the luminal surface may be exploited to identify transforming/cancerous tissue, in particular with traditional immunohistochemistry of sectioned pathologic tissue or our recent work developing dynamic biochemical tissue analysis,^{10,56} these are beyond the scope of this review. Note that we use the term “transforming/cancerous tissue” to encompass the full range of dysplastic to malignant tissue.

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Signature molecules (moieties whose expression is increased on transforming/cancerous tissue relative to normal tissue) overexpressed on two dominant types of esophageal cancer, adenocarcinoma and squamous cell carcinoma, as well as dysplastic and neoplastic areas in the esophagus are summarized in Table 1. Among the molecular probes presented in Table 1, Bevacizumab,⁹⁴ EMI-137,⁹⁵ ASYNYDA,^{96,97} and SRRPASFTARE⁹³ were used to detect transforming esophageal tissue in clinical trials. In addition, Anti-EGFR mAb,⁹⁸ Wisteria floribunda,⁹⁹ and QRHKPRE⁹² were utilized for the detection of transforming colon tissue in clinical trials (Table 1). Additionally, several cancer stem cell surface markers, indicative of a rare subset of cancer cells that possess the capacity to initiate tumor formation, for esophageal cancer are reported in Table 1. Furthermore, several investigators have characterized pathological esophageal tissue with functional molecular probes utilizing various classes of molecules including small molecules, peptides, proteins, monoclonal antibodies (mAbs), lectins, glycolipids, or glycans (Table 1).³⁹ These studies have revealed alterations in the biochemistry of the transforming tissue but not the signature molecule(s) underlying the altered biochemistry. This work includes the identification of: wheat germ agglutinin (WGA) as a “negative contrast agent” staining healthy tissue to a higher level than dysplastic tissue⁴; the peptide SNFYMPL which exhibited preferential binding to cultured esophageal adenocarcinoma cells (OE33) and a high level of binding to dysplastic tissue⁵⁰; and the peptide, ASYNYDA, specific for Barrett’s neoplasia.⁷⁷

Within the colon, the signature molecules that have been reported to be overexpressed on colorectal transforming/cancerous tissue compared to normal tissue, and cancer stem cell markers for colorectal cancer are presented in Table 1. Kuo *et al.*⁴⁴ characterized pathological colorectal tissue with functional molecular probes and distinguished normal epithelial tissue from low-grade dysplasia, high-grade dysplasia, or carcinoma using WGA and helix pomatia agglutinin (HPA). Hsiung *et al.*³² reported that the VRPMPLQ peptide displayed a preferential binding to colonic dysplasia *in vivo*. Joshi *et al.*³⁸ determined that KCCFPAQ targets the cell surface of sessile serrated adenomas cells with a V600E BRAF point mutation. These studies revealed alterations in the biochemistry of the transforming tissue using probes (i.e. the lectins and peptides) but not the signature molecule(s) underlying the altered tissue surface biochemistry. A list of molecular probes for colorectal cancer and their signature molecule targets is shown in Table 1.

With the identification of this library of candidate signature molecules, and molecular probes that recognize transforming tissue even if the exact signature

molecule(s) is not known, the opportunity arises to exploit these changes as targets for the identification and characterization of neoplastic tissue *via* MEI.

MOLECULAR ENDOSCOPIC IMAGING (MEI)—AN EMERGING TECHNOLOGY FOR DETECTING AND CHARACTERIZING TRANSFORMING/CANCEROUS TISSUE

The primary procedure for identifying neoplastic alterations in the colon and the esophagus is endoscopy. While there is a plethora of endoscope designs, they all share common features. The distal tip of the endoscope, i.e. the end of the endoscope that is inserted into the GI tract, has: a camera which sends images of the tissue under observation to a monitor and often-times to a recording device; a light source(s) for bright field and fluorescence illumination; a water jet used to wash the tissue at a range of volumetric flow rates; a way to introduce air to expand the luminal opening; and a spray catheter that can be used to introduce various solutions. For a complete overview of endoscopic techniques (e.g. white light endoscopy and autofluorescence imaging) the reader is directed to several excellent reviews.^{21,31,78} The majority of these techniques focus on microanatomical changes (e.g. abnormal microstructure of the cell surface and altered microvascular architecture) that often accompany transformation.⁸³ In contrast, MEI is an advancing technology that seeks to exploit the signature molecules accompanying transforming/cancerous tissue to discriminate between normal and pathological tissue and to characterize pathological tissue.²³ These signature molecules can serve as targets for the identification of transforming tissue *via* molecular probes cognate to the target moiety, i.e. ligands. A search of the United States Patent and Trademark Office website (www.uspto.gov) for filed patents including the terms “molecular”, “endoscope”, “endoscopic”, “imaging”, “probe”, and “tissue” resulted in 684 hits, while a search of patents filed worldwide using the same six terms by Google Patents produced 1487 results [e.g. US20170246324A1, US20100061929A1, US8901276B2, US9456794B2, US20100310459A1, US20180263475A1, AU2009223235B2]. Clearly, based on the large number of patent filings, there is significant interest in designing tools using MEI and applying these tools for clinical applications. As pointed out in the above section, certain ligands specific for transforming/cancerous tissue have been identified even though the underlying signature molecule(s) are not known. Regardless of whether the signature molecule is known, the ligands form the basis for the design of “probes” to investigate the biochemical nature of the tissue *via* MEI. As summarized by Joshi and Wang,³⁹ numerous MEI

TABLE 1. Signature molecule, cancer stem cell marker, and molecular probe candidates for targeting esophageal and colorectal transforming/cancerous tissue using molecular endoscopic imaging (MEI).

Esophageal cancer	Signature molecules for different stages/types of cancer		Cancer stem cell markers		References	Molecular probe/target	References
	Transforming/premalignant (dysplasia/neoplasia)	HER2 c-Met FGFR2 VEGF EGFR	HER2 c-Met CD90 CD271 EpCAM ITGA7	CD44 CD54 CD90 CD271 EpCAM ITGA7			
Esophageal cancer	Transforming/premalignant (dysplasia/neoplasia)	HER2	HER2	CD44	91	ASYNVDA/a*	77,96,97
		c-Met	c-Met	CD54	82	SNFYMPL/a	50
		FGFR2	FGFR2	CD90	81	Recombinant E-selectin/sLe ^A & sLe ^x	59
		VEGF	VEGF	CD271	34	Anti-CD44v5 mAb/CD44v5	59
		EGFR	EGFR	EpCAM	76	EMI-137/c-Met	95
	Adenocarcinoma	EGFR	EGFR	ITGA7	57	QRHKPRE/EGFR	103
		TGM2	TGM2		16,84	SRRPASFRITARE/FGFR2	93
		sLe ^A	sLe ^A		49	Bevacizumab/VEGF-A	47,58,94
		sLe ^x	sLe ^x		5,59	KSPNPRF/Her2	104
		FGFR2	FGFR2		59		
Colorectal cancer	Squamous cell carcinoma	CD44	CD44	CD44	18		
		EpCAM	EpCAM	EpCAM	30		
		sLe ^A	sLe ^A		62		
		sLe ^x	sLe ^x		55		
		ABCG2	ABCG2		33		
	Transforming/premalignant (polyps)	CD44v5	CD44v5		59		
		c-Met	c-Met		63,88,89		
	Cancerous	HER2	HER2	CD26	64	VRPMPLQ/a*	32,100
		VEGF-A	VEGF-A	CD44	17	KCCFPAQ/a*	38,101
		EGFR	EGFR	CD58	87	QRHKPRE/EGFR*	92,102
		CLDN1	CLDN1	CD133	60,68	Wisteria floribunda/ disaccharide LacdiNAc*	27,99
		LY6G6D/F	LY6G6D/F	CD166	17	Anti-EGFR mAb/EGFR*	52,98
Colorectal cancer	Cancerous	TLR4	TLR4	EpCAM	17	EMI-137/c-Met	8,106
		LGR5	LGR5		79	Bevacizumab/VEGF-A	47,105
		sLe ^A	sLe ^A		67	KSPNPRF/Her2	37,104
		sLe ^x	sLe ^x		67		
		HER2	HER2		37,66		
	Transforming/premalignant (polyps)	ERBB3	ERBB3		66		
		c-Met	c-Met		8		
	Cancerous	HER2	HER2	CD26	64	VRPMPLQ/a*	32,100
		VEGF-A	VEGF-A	CD44	17	KCCFPAQ/a*	38,101
		EGFR	EGFR	CD58	87	QRHKPRE/EGFR*	92,102
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		sLe ^x	sLe ^x		67		
		HER2	HER2		37,66		
	Transforming/premalignant (polyps)	ERBB3	ERBB3		66		
		c-Met	c-Met		8		
	Cancerous	HER2	HER2	CD26	64	VRPMPLQ/a*	32,100
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		EGFR	EGFR	CD58	87	QRHKPRE/EGFR*	92,102
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		LY6G6D/F	LY6G6D/F	CD166	17	Anti-EGFR mAb/EGFR*	52,98

Signature molecules for different stages/types of cancer are given in column 3. Cancer stem cell markers and molecular probes/targets for esophageal and colorectal cancer are given in columns 5 and 7 respectively.

*The probes are introduced via an endoscope.

a Target is unknown.

FIGURE 1. The general protocol for molecular endoscopic imaging (MEI). (a) The GI tract is lined with a mucosal layer (depicted as a grey wavy line). In the esophagus, this layer, if present, is relatively small while in the colon it is quite significant. This mucus layer imposes a barrier that the probes would need to cross to interact with the surface of the epithelium. (b) To remove the mucus layer and expose the epithelial surface and signature molecules, a suspicious site is washed (perhaps with a mucolytic agent) with the water jet of the endoscope. (c) The probes are delivered at the suspicious site *via* the spray catheter of the endoscope and allowed to incubate with the tissue. (d) Unbound probes are washed away *via* the water jet of the endoscope prior to detection. (e) The tissue is examined *via* the endoscope to quantify the presence of the probes.

schemes have been tested in clinical trials. (Note that although the probes could be introduced in ways other than *via* an endoscope, e.g. oral administration, we limit our discussion to protocols wherein the probes are introduced *via* an endoscope). These trials include the following identified by the trial's targeting ligand: ASYNYDA^{96,97} for the esophagus, and VRPMPLQ,^{32,100} KCCFPAQ,^{38,101} QRHKPRE cognate to EGFR,^{92,102} *Wisteria floribunda* cognate to the disaccharide LacdiNAc (β -D-GalNAc-[1 $\rightarrow$ 4]-D-GlcNAc),^{27,99} and anti-EGFR mAb^{52,98} for the colon.

The general protocol for MEI, wherein the probes are introduced *via* an endoscope, is depicted in Fig. 1 and is as follows.^{59,77,85,96–102} A suspicious site is washed *via* the water jet of the endoscope to remove the mucus layer and expose the epithelial surface. [While beyond the scope of this review, we would like to point out that the mucus layer itself could be used directly for diagnostic and prognostic cancer screening by

evaluating the expression of mucins, or other molecules, reported to be involved in the development of cancers of the gastrointestinal tract.^{20,36,43}] In the esophagus, the mucus layer is limited⁶¹ but it is quite pronounced in the colon.^{14,19,46} Thus, a mucolytic agent may be required during the wash to help expose the epithelial layer of the colon. A high-flow rate (10.4 mL/s) water jet works better for colon pre-washing³ to remove mucus. Note that nanoparticles have been designed to penetrate the mucus layer (e.g. Refs. 45 and 90) and such technology could be used for MEI to avoid washing or if washing is insufficient to remove the mucus layer. Subsequent to the pre-wash, the probes are delivered at the suspicious site *via* the spray catheter of the endoscope. Following administration, the probes are allowed to incubate with the tissue, and the tissue subsequently washed to remove unbound probes. The tissue is then examined *via* the endoscope camera to quantify the presence of the

probes. Detection of the probes indicates expression of the signature molecule(s) and suggests the presence of transforming/cancerous tissue. In this manner, identification of transforming/cancerous tissue can be achieved *via* MEI. Note that when ligand conjugated particles are utilized, the examination of the tissue might take place under controlled fluid flow conditions to reveal the nature of the adhesion (e.g. rolling adhesion or firm adhesion)^{56,59} of the particles to the tissue.

The Biophysics of MEI

While the scenario outlined above may seem straight forward, the biophysics underlying such assays is rather complex. The success or failure of the assays depends, in large part, on the degree to which the probes exhibit selective recognition of transforming/cancerous tissue relative to normal tissue. At the fundamental level, selective recognition will be dictated by the convective/diffusive mass transport of the probes and the “interactions” of the probes with the target and normal tissues. Both of these factors depend on the design of the probe. The probe could be the ligand itself linked to a detector molecule (e.g. a fluorophore) or the ligand conjugated to a particle. We refer to the former as unconjugated ligands and the latter as conjugated ligands. The unconjugated ligands will likely undergo higher levels of diffusive mass transport relative to the larger conjugated ligands. The elevated diffusive transport will enhance the chances of the unconjugated ligands encountering their target. This factor may be quite important in the colon where a thick mucus layer is present which impedes access to the target epithelial cells.^{14,19,46} Additionally, due to their relatively small size, unconjugated ligands may be able to cross the epithelial layer either through diffusion, passive or active transport. This feature may be necessary if the target is intracellular or lies beyond the epithelial lining, rather than present on the luminal epithelial surface ideal for MEI. Notwithstanding the salient features of unconjugated ligands, these probes have several limitations including the facts that: (i) the level of signal is limited, in large part, by the number of target signature molecules on the tissue and the number of detector molecules that can be coupled to the unconjugated ligand, (ii) the unconjugated ligand may non-specifically cross the cell membrane and epithelial layer and enter non-target cells which may increase non-specific signal and off-target distribution, (iii) the design parameters for the unconjugated ligand is relatively limited, and (iv) simultaneous targeting of multiple signature molecules *via* multiple ligands, while not impossible, is difficult.¹ Thus, many investigators have focused on the use of conjugated ligands for

detection and characterization of transforming tissue within the GI tract [e.g. Refs. 11, 35, 41, 59, and 85].

For probing the esophagus and the colon with conjugated ligands, it is important to recognize that the luminal diameter of these organs is on the order of centimeters which is in marked contrast to the vasculature where the small size of the capillaries ($\sim 2 \mu\text{m}$ in diameter) can place an upper limit on the size of particle that can be used for targeting. Thus, it is possible to utilize particles ranging in size from nanometers to micrometers (or larger) in a detection scheme focused on the esophagus and colon. Indeed, gastroesophageal reflux disease (GERD) is often investigated with gel-cap-sized probes (significantly larger than microparticles) implanted in the esophagus.⁹ Particles with smaller diameters ($\sim < 0.5 \mu\text{m}$) may be endocytosed⁸⁶ and nanometer sized particles would undergo significant diffusive mass transport which could facilitate or, alternatively, confound the assay in ways described above for unconjugated ligands. As we've pointed out previously,⁵⁹ micron-sized particles have several salient features including the facts that they (i) allow a relatively large volume of detector molecules (e.g. a fluorophore) to be carried per particle [the volume is proportional to the particle radius cubed ($V \propto r^3$)], (ii) can often be detected utilizing bright field microscopy, (iii) are readily washed out subsequent to detection, (iv) should exhibit limited, if any, non-specific uptake by cells thus reducing off target distribution and non-specific signal, and (v) are relatively easy to manipulate experimentally. Thus, in developing a targeting detection scheme, a relatively wide range of particle sizes is possible, and several factors (e.g. detection scheme, minimizing non-specific signal and off target distribution⁵⁹) need to be considered when selecting the size.

The above paragraphs linked the diffusive transport to the probe design. More broadly, the probe transport will be a function of a multitude of factors and a fairly comprehensive list of these includes: (i) whether the probe is an unconjugated ligand or a ligand conjugated to a particle and the size²⁸ (as described above); (ii) the molecular weight of the ligand and charge of the probe²⁸; (iii) the fluid dynamics operative during the introduction of the probes and the wash step to remove unbound probes^{12,28}; (iv) the properties of the fluid (e.g. viscosity) present during the assay^{12,28}; and (v) the presence and nature of the mucus layer that overlies the epithelium [more pronounced in the colon than the esophagus^{14,19,46,61}]. The former factors are determined by the design of the probe. The operative fluid dynamics can be determined from first principles utilizing details of the design of the spray catheter/water jet, the volumetric flow rate and the geometry of the segment of GI tract of interest. In regard to the physical properties of the fluid layer and the nature of

FIGURE 2. Schematic of probes interacting with esophageal/colonic epithelium. At sites of transformation (right two panels), the epithelium may express signature molecules that can serve as targets for probes seeking to identify transforming/cancerous tissue. The probes consist of molecules cognate to the signature molecules, i.e. ligands, which can be unconjugated (top two panels) or conjugated to particles (bottom two panels). The goal of MEI is to design probes and a delivery system that allows *in situ* discrimination between healthy and transforming/cancerous tissue. The general protocol is described in Fig. 1. For the case of probes consisting of ligands conjugated to particles of appreciable size, the particles will experience a force and torque exerted on the particles by the local flow (depicted in lower right-hand image). The relative strengths of the disruptive force/torque and the adhesive force determines if the particulate probes will adhere to the epithelium and the nature of the adhesion.

the mucus layer, there is a considerable body of literature related to these issues which could be brought to bear (see Refs. 14 and 71 for detailed review).

Transport of the probes to the luminal surface provides the opportunity for the probes to “interact” with the tissue (Fig. 2). For the case of unconjugated ligands, or ligands conjugated to small nanometer sized particles, standard deposition kinetic modeling (see Lok *et al.*⁵³ for further details) may suffice to characterize the interactions of the probes with the tissue.⁵³ However, for the case of probes consisting of a ligand conjugated to larger particles (micron or greater sized particles), the “adhesive interactions” between the probe and the tissue are fairly complex.^{28,29} That said, larger ligand-conjugated particles adhering to the GI epithelium is in many ways analogous to the problem of cell adhesion to the vascular endothelium which has been studied extensively.^{2,12,15,28,29,42,48} Thus, cell adhesion provides a framework to analyze MEI schemes that utilize probes consisting of ligands conjugated to larger particles. Applying such a framework leads to the following. By definition, a ligand bearing particle firmly adherent to a segment of the epithelium exhibits zero velocity. Since the velocity is not changing with time, the particle is not accelerating and thus the sum of the disruptive and adhesive forces and torques acting on the particle are balanced. If a fluid stream is being applied to the adherent particle, the particle will experience a disruptive force due to the force of the fluid on the particle.^{26,28,29} The particle will also experience a disruptive torque due to the fluid shear.^{26,28,29} This disruptive force/torque is

countered by an “adhesive force” at the interface of the tissue and the adherent particle.^{28,29} The adhesive force is the combination of “non-specific” attractive/repulsive forces between the particle surface and the tissue and the, ideally dominant, “specific force” mediated by the bonds formed between the ligand present on the particle and the target signature molecule.^{28,29} Since the aggregate of the non-specific forces can be attractive, i.e. pro-adhesive, particles can bind to tissue independent of specific ligand—signature molecule bonds. This non-specific adhesion may result in off-target tissue recognition which will diminish selective recognition of the target tissue. That said, non-specific forces are relatively weak and typically yield only “background” adhesion. In contrast, the specific forces, due to the bonds between the ligand and the target signature molecule, are relatively strong. An aggregate of these specific bonds can counter the disruptive force/torque due to the flow of the fluid and thus the particle can remain firmly adherent even under relatively high shear stress conditions.²⁸ Importantly, the disruptive force/torque, due to the fluid flow, can help discriminate between non-specific adhesion and specific adhesion, i.e. the disruptive force can be set to remove particles only bound by non-specific forces but insufficient to disrupt particles adherent *via* specific adhesive bonds.

While the above discussion provides a relatively straightforward analysis of desired specific vs. non-specific interactions, work from the field of cell adhesion clearly indicates that (i) a variety of “adhesive states”¹² are possible for the ligand-conjugated probe

FIGURE 3. The selectivity equation. In general, the goal of MEI schemes is to maximize the distinction between transforming/cancerous tissue and healthy tissue, i.e. to achieve maximal selectivity. At the most fundamental level, selectivity will be dictated by the convective/diffusive transport of the probes and the “deposition/adhesive interactions” of the probes with the tissue. The transport and the “interaction” of the probes will be determined by a variety of factors which can be categorized into target tissue parameters [Item I in the figure], probe design parameters [Item II in the figure], and delivery design parameters [Item III in the figure]. The former set of factors are essentially fixed, while the latter two sets of factors can be controlled and manipulated. The exact design chosen, i.e. the parameters stipulated in II and III, strongly influences the operative biophysics and thus has a significant impact on the ultimate selectivity of the assay. The goal of engineering MEI is to achieve an assay design that yields maximal selectivity. Previous work in cell adhesion [e.g. Refs. 2, 12, 15, 28, 29, 40, 42, 48, and 73], and those that bridge MEI and cell adhesion,⁵⁹ can provide key insights into this rather complex problem.

particle including firm adhesion described above and a “rolling” adhesion where the probe moves in the direction of flow but at a reduced velocity due to adhesive interactions,²⁵ and (ii) the ligand-signature molecule bonds mediating the specific adhesion will respond to the applied force.^{2,28} The response of specific adhesive bonds to applied force has also been studied extensively in the field of cell adhesion (e.g. Refs. 2, 12, 28, and 42 and reviewed in Ref. 13) and provides insights. Utilizing this well-established framework, the ligand—signature molecule bond formation/dissociation can be modeled as a reversible chemical reaction.²⁸ This reaction will have an intrinsic kinetic on rate and an intrinsic kinetic off rate.²⁸ The kinetic rate, however, will be coupled to the stress applied to the bonds.^{28,42} For example, the greater the force applied to a “slip” bond the more rapidly the bond will break which is reflected in an increased off rate.²⁸ The balance between the adhesive force, dictated, in part, by the intrinsic kinetics and the bonds’ response to applied force, and the disruptive force and torque due to the flow of the fluid, will determine whether the ligand-conjugated particle probe will adhere to the tissue and the nature of the adhesion, e.g. firmly adherent or rolling.^{12,28}

Engineering MEI

The prior sections clearly illustrate that the ability of the probe to selectively recognize transforming/cancerous tissue vs. normal tissue will be influenced by a plethora of factors. These factors can be

grouped into subsets as follows: (I) target tissue parameters, (II) probe design parameters, and (III) delivery design parameters (Fig. 3). The first of these is dictated by the biology/pathology and are essentially fixed, while the latter two can be engineered to obtain optimal selectivity. Regardless of the details of a given design, the goal remains the same: maximize specific recognition of target tissue and minimize recognition of off-target tissue, i.e. maximize selective recognition. Figure 4 gives a flow chart to engineer schemes that achieve maximal selective recognition.

As depicted in Fig. 4, the process starts with a detailed characterization of transforming/cancerous tissue relative to normal tissue. This analysis has yielded, and will continue to yield, (i) ligands that bind to neoplastic tissue but whose cognate signature molecule is not known and (ii) signature molecules. The identification of target signature molecule(s) leads to the opportunity to develop a molecular ligand(s) (i.e. a moiety cognate to the target signature molecule) that selectively identifies the target. As mentioned previously in this review, these molecular ligands could be small molecules, peptides, proteins, mAbs, lectins, glycolipids, or glycans. From these options, the ligand(s), and if known, the target signature molecule(s), are selected. Next, the detection scheme needs to be determined, and this decision impacts the further design of the probe. Wang *et al.*⁸⁵ have provided proof-of-principle for detecting targeted Raman scattering nanoparticles *via* a spectral endoscope. Ligands conjugated to particles with sufficient size (i.e. micron size) could be detected under white light illumination using

FIGURE 4. Engineering MEI. This figure depicts a flow-chart for engineering MEI. As shown, the process starts with a detailed characterization of the biochemistry of transforming/cancerous tissue relative to normal tissue. The goal of this effort is to identify signature molecules or ligands that recognize pathological tissue to a greater extent than normal tissue despite lacking knowledge of the underlying signature molecule (purple rectangles in figure). If the signature molecule is known, ligands cognate to the signature molecule can be developed (dark green rectangle). The various classes of molecules from which such ligands may be derived include the ones listed in the lighter green rectangles to the right. For ligands where the underlying signature molecule is not known, the ligand is typically a peptide or a lectin. With the identification of ligands and signature molecules, the decision of the targeting chemistry can be made. (The decision points are depicted in yellow diamonds in the figure.) Next the mode of detection of the probes needs to be considered. We've broadly divided possible detection techniques into three classes (orange rectangles): Raman spectroscopy, fluorescence (including confocal, fiber optic, and near infra-red), and white light microscopy. Utilization of Raman spectroscopy necessitates the probe being made of Raman scattering particles while utilization of white light microscopy necessitates a probe in the micrometer, or larger, size range. If fluorescence is the planned mode of detection, then a range of options are available including unconjugated ligands tagged with a fluorophore and ligand-conjugated nanoparticles/microparticles containing a fluorophore. For both bright field and fluorescent detection schemes, the particles that could be used include biodegradable particles,^{24,69} polymersomes,^{22,51} liposomes,⁷⁵ and quantum dots⁷ (depicted in the blue rectangles). While it is convenient to depict the design process as linear, as we have done here, in reality there is cross-talk between the various stages of the design process. Note that in this schematic, we have restricted our discussion to techniques wherein the delivery and imaging of the probes is done *via* an endoscope. Other delivery methods e.g. oral and intravenous administration, and detection techniques, e.g. MRI, are possible but are beyond the scope of this review.

a high magnification endoscope. Detection of unconjugated ligands, and enhanced recognition of conjugated ligands, could be achieved *via* a fluorophore linked to the ligand or contained within the particle to which a ligand is conjugated. Thus, possible detection schemes include bright field microscopy, fluorescence microscopy [including confocal,³² fiber optic,¹⁰² and near infra-red⁵⁸], and Raman spectroscopy. For

Raman spectroscopy, Raman scattering particles would be used.⁸⁵ In other cases, if the probes are to be ligand-conjugated particles, there are several options for the composition of the particles including nano/micron sized biodegradable particles,^{24,69} polymersomes,^{22,51} liposomes,⁷⁵ and quantum dots.⁷ Finally, with particles it would be quite easy to utilize multiple ligands, to one or more targets, on a single particle and

to tune the surface density of ligands on the particles to maximize selective recognition.

CONCLUSION

There is an ever-expanding library of knowledge regarding the biochemical alterations that accompany cancer progression in the esophagus and colon. This knowledge can be exploited to design MEI schemes that afford *in situ* detection of transforming/cancerous tissue. The success of these assays relies on the selective binding of probes to transforming/cancerous tissue relative to normal tissue. This selective recognition is determined by a plethora of factors that interact in a rather complex manner. That said, several of these factors can be tuned to achieve high levels of selective recognition by utilizing techniques and analyses developed from the study of cell adhesion [e.g. Refs. 2, 12, 15, 28, 29, 40, 42, 48, and 73]. An engineering approach bridging MEI and cell adhesion,⁵⁹ can facilitate meeting this daunting challenge.

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CONFLICT OF INTEREST

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