Early Lesions in Experimental Bladder Cancer: Scanning Electron Microscopy of Cell Surface Markers

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Summary

The administration of N-[(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT) at a dose of 0.2% of the diet to male Fischer rats results in the appearance of urinary bladder epithelial lesions progressing from hyperplasia to invasive transitional cell carcinomas. These progressive epithelial alterations have been observed by scanning electron microscopy at 2-week intervals with special attention paid to cells covering the luminal surface. After 2 to 4 weeks of FANFT administration there is mild pleomorphism and moderate swelling of the surface cells giving a cobblestone appearance. The normal pattern of microridges of superficial transitional cells is still present, although occasional cells by 4 weeks of FANFT feeding are covered by small uniform microvilli. By 6 weeks these changes are more extensive and more cells are covered with uniform microvilli. By 8 weeks, cells covered with pleomorphic microvilli are found in discrete foci, and by 10 weeks nodular or papillary lesions are present. The 2- and 4-week lesions regress to normal within 2 to 4 weeks of discontinuing FANFT administration, and the 6- and 8-week lesions regress toward normal in 4 to 6 weeks of being fed control diet. The 6-week lesions have regressed entirely to normal 44 weeks after FANFT was stopped, but the 8-week lesions have progressed to moderate or marked hyperplastic lesions by 42 weeks of control diet.

Materials and Methods

Experimental design and fixation of the urinary bladders for light microscopy and TEM are given elsewhere in this issue (3). Briefly, FANFT was administered to weanling male Fischer rats at a dose of 0.2% of the diet for 2, 4, 6, 8, 10, 12, 14, or 20 weeks, and rats from each group were examined at 2-week intervals through 20 weeks, and again at 50 weeks for urinary bladder lesions. SEM specimens, approximately 5 x 5 mm were rinsed briefly in 0.1 M cacodylate buffer (pH 7.4), postfixed in 1% OsO₄ 0.1 M cacodylate buffer (pH 7.4), and rapidly dehydrated through an ascending alcohol series; they were placed in a Denton DCP-1 critical-point drying apparatus (Denton Vacuum, Inc., Cherry Hill, N. J.) and dried by the method of Anderson (1). Specimens were cemented to specimen stubs, coated with carbon followed by 150 A of gold and examined at 25 kV in either a JEOL Model JSM-U3 or an ETEC Autoscan SEM.

Results

Normal bladder surface epithelium examined in the SEM is flat (Fig. 1) and demonstrates a surface covered by flat polygonal cells of relatively uniform size (Fig. 2). The luminal surfaces of these cells are covered with a complex network of microridges (Fig. 3). Tight cell junctions (zonulae occludentes) between lateral borders of cells appear as a fine line composed of fused microvilli. The subepithelial blood vessels in normal, fully inflated bladders appear as smooth elevations running across the specimen. By TEM the luminal surface membrane is made up of asymmetrical 11-nm concave plaques that appear as troughs and are
joined by crests of symmetrical 9- to 10-nm interplaque membrane (Fig. 3, inset).

Slight changes in surface topography can be found by the 2nd week of FANFT feeding, with foci of flat polygonal cells varying in size. The cells are covered with microridges and the topography, although flat, has slight elevations compared to the normal surface.

By 4 weeks of FANFT feeding, the surface displays greater variability in cell size with varying degrees of elevation of cells giving a “bumpy” cobblestone appearance to the surface (Fig. 4). Small uniform microvilli are found on the renewal intermediate cells occasionally seen replacing exfoliated superficial cells. By TEM, microvilli may be covered by both asymmetrical plaques and symmetrical unit membrane, but the tips of microvilli are always made up of symmetrical membrane. A fuzz is evident on the microvilli, presumably a glycocalyx.

With 6 weeks of FANFT feeding there is a diffuse cobblestone appearance to the surface (Fig. 5). Surface cells in many areas are round, rather than polygonal, and the rounded cells have microridges and some uniform microvilli (Fig. 5, inset). The areas of increased cell growth, as evidenced by a cobblestone appearance, are found in the epithelium overlying subepithelial blood vessels. By TEM, many microvilli are found, particularly on the smaller, rounded cells, and there appear to be fewer asymmetrical plaques present.

With 8 weeks of FANFT feeding, cobblestoning is more widespread and surface cells are pleomorphic, many of them being small and round (Fig. 6). The cells in the cobblestone areas are covered with both microridges and microvilli, and the small, round cells have pleomorphic microvilli without microridges (Fig. 6, inset). At 10 weeks, papillary and nodular lesions are present consisting of cells that are either round or teardrop in shape with numerous pleomorphic microvilli. Few flat, superficial cells are present in these lesions. By TEM, surface cells lining the bladders of rats fed FANFT for 8 and 10 weeks have fewer asymmetrical plaques than do control bladders on the luminal membrane, and microvilli become more frequent.

The changes are more marked at 12, 14 (Fig. 7), and 20 weeks of FANFT feeding. By SEM, the entire bladder surface appears cobblestoned with papillary and nodular aggregates of cells protruding into the lumen. These tumors become larger as the experiment progresses (Fig. 8). The cells in the flatter areas on the mucosal surface are pleomorphic (Fig. 9), and most of them have either a rounded (Fig. 10) or a leafy-appearing microridge system (Fig. 11) on their luminal surfaces. Occasional smaller, round cells with pleomorphic microvilli are also present on the flat areas. The tumors, however, have a predominance of these small, round cells with pleomorphic, often bizarre (Fig. 12) microvilli. These microvilli have blunt rounded contours at their tips as visualized by TEM and are quite different from the long, slender, pointed tips of the microvilli-like structure of the leafy and rounded microridges.

It has been demonstrated previously that rats fed FANFT for 20 weeks and then control diet for an additional 30 weeks developed large bladder tumors with microinvasion of the subepithelial connective tissue or invasion of the muscle layers. The surface of the invasive tumors is covered with pleomorphic cells with pleomorphic microvilli covered with a glycocalyx fuzz, the microvilli becoming increasingly bizarre from 20 to 50 weeks of the experiment.

The bladder mucosa of rats fed FANFT for 2, 4, or 6 weeks and then fed control diet returns to normal. This process of normalization occurs within 2 weeks in 2-week-FANFT-fed rats. Rats fed FANFT for 4 weeks are near normal after 2 weeks of control diet, but small foci of cells with slight variability in size and shape are still detectable, these cells being covered with microridges except at cell junctions where microvilli are present as in control rats. Such foci are still present after 16 weeks on control diet, but the surface appears entirely normal by the 50th week of the experiment and resembles the surface seen in Fig. 2. Rats fed FANFT for 6 weeks appear to regress like the 4-week-fed rats once they are placed on control diet, except that it takes 4 weeks instead of 2 for the entire surface to be covered by polygonal superficial cells with microridges. Pleomorphic microvilli never become apparent. All of these rats except 1 had normal-appearing surfaces by the 50th experimental week, the exception consisting of a minute papillary projection into the lumen of 1 rat bladder that had large, uniform polygonal surface cells covered with microridges.

The bladder surface alterations of rats fed FANFT for 8 or 10 weeks also tend to regress once the rats are fed control diet, but they never become normal and eventually progress. Those fed FANFT for 8 weeks have bladders with cobblestoned surface appearance after 2 or 4 weeks of control diet, with the cells being variable in size and shape, but occasional foci of small, round cells with either uniform or pleomorphic microvilli are detectable by SEM. Although most of the bladder surface becomes flatter and the cells more uniform with control diet fed for 6 to 12 weeks, occasional round cells with microvilli can still be detected. After 42 weeks of control diet (50 weeks of experiment), the rats had moderate to marked hyperplastic nodules of the bladder with numerous small, round cells covered by pleomorphic microvilli. The areas of the bladders not involved with nodular lesions were nevertheless abnormal with a cobblestone appearance (Fig. 13) and mild pleomorphism of polygonal cells covered with microridges and microvilli (Fig. 14). Rats fed FANFT for 10 weeks and then control diet had a similar pattern of regression, but at a slower rate and with more microvilli-covered round cells present at each time interval as compared to the 8-week-FANFT-fed rats. By 10 weeks on control diet there was still extensive cobblestone appearance, and the surface cells were varied in size and shape with numerous cells covered with pleomorphic microvilli. By the 50th week of the experiment all of the rats that had received FANFT for 10 weeks had tumors present in the bladder, one of which had invaded through the muscular layers. The surface cells of the tumors were markedly pleomorphic and were covered with microvilli, frequently bizarre in appearance. The nontumorous areas had large, flat cuboidal cells on the surface with leafy microridges and/or microvilli.
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The bladders of animals fed FANFT for 12, 14, or 20 weeks did not show regression of their lesions when placed on the control diet. When examined at the 50th week of the experiment, tumors had developed as described above and the surface was covered with pleomorphic cells, most of which were small, round cells covered with pleomorphic microvilli.

Discussion

The surface of the normal rat urinary bladder is composed of large, flat, polygonal superficial cells of rather uniform shape and size. The luminal membrane is composed of symmetrical membrane connecting plaques of unique composition which appear as asymmetrical membrane by TEM. The interconnecting areas of symmetrical membrane appear as microridges when viewed by SEM. The intermediate and basal cells, however, do not have a microridge system nor do the nonluminal surfaces of superficial cells. Instead, relatively uniform microvilli cover their surfaces.

With the administration of FANFT for 2 to 6 weeks there is a gradual enlargement and proliferation of the bladder epithelial cells resulting in a "bumpy" or cobblestone appearance and slight to moderate variation in the size and shape of the cells. On the luminal surface of the cells the system of microridges is retained. Occasionally, foci are seen at 4 to 6 weeks where the normal superficial cells have exfoliated into the urine and the underlying intermediate cells forming the new luminal surface are visualized with uniform microvilli on their surfaces. If FANFT is withdrawn at this time the lesions gradually return to normal, indicating that the abnormalities present through 6 weeks represent nonspecific, nonneoplastic hyperplasia.

After 8 weeks of FANFT administration there is continuation of the changes that were present at 6 weeks, but in addition there is the 1st appearance on the luminal surface of microvilli that vary in thickness and in length instead of the uniform microvilli normally present on intermediate cells. Two pieces of evidence suggest that these pleomorphic microvilli may reflect an irreversible alteration of the bladder mucosa: (a) microvilli increase in number and degree of pleomorphism with the progression of the epithelial lesions until the luminal surface of invasive tumors is covered with many pleomorphic, often bizarre, microvilli; (b) pleomorphic microvilli, once they appear at the 8th week, are subsequently present for the remainder of the experiment on the luminal surfaces of at least some epithelial cells. Although most of the altered bladder epithelium of rats fed FANFT for 8 weeks has returned almost to normal by 20 weeks, foci of small, round cells with pleomorphic microvilli on their luminal surface are detectable in the epithelium by SEM from then on during the experiment, even though by light microscopy the lesions appear identical to the mild hyperplasia seen immediately after 2 to 4 weeks of FANFT administration. Despite this initial regression, however, these lesions in rats fed FANFT for 8 weeks eventually progress to moderate or marked hyperplasia at the 50th week of the experiment with the luminal cell surfaces covered with pleomorphic microvilli. Whether or not these hyperplastic lesions after 8 weeks of FANFT feeding will progress to carcinoma after more than 50 weeks is being investigated.

On the other hand, rats fed FANFT for 10 weeks followed by control diet have surface cells covered with pleomorphic microvilli and the lesions eventually progress to invasive carcinomas. This type of microvilli represents irreversibility in our system. By contrast, lesions from rats fed FANFT for 6 weeks do not have pleomorphic microvilli on their surface cells, and eventually the epithelium returns to normal. The single lesion present at 50 weeks in rats fed FANFT for 6 weeks was papillary, but its surface was comprised of large, rather uniform polygonal cells with microridges. There was no evidence of cellular anaplasia or invasion by light microscopy.

Pleomorphic microvilli have also been demonstrated in other experimental bladder cancer models (2) and in human bladder carcinomas (4). At this time, however, it is not clear whether, in other experimental systems or in human material, pleomorphic microvilli are specific for neoplastic transformation or whether they might also represent reversible proliferative change.

References

Fig. 1. Low magnification of normal rat urinary bladder with flattened surface and subepithelial blood vessels causing slight elevations of the overlying epithelium. × 30.

Fig. 2. Normal rat bladder with flat, uniform polygonal superficial cells. × 200.

Fig. 3. Normal rat bladder with microridges on luminal surface of superficial cells. × 10,000. Inset, TEM of normal rat bladder demonstrating cross-sectional view of microridges. × 6,000.

Fig. 4. Variation in size and slight elevation of cells into the lumen of a mildly hyperplastic bladder from a rat fed FANFT for 4 weeks. × 3,000.

Fig. 5. More pronounced pleomorphism of surface cells to cobblestone appearance of surface from a rat fed FANFT for 6 weeks. × 1,000. Inset, occasional small round cells have uniform microvilli. × 8,000.

Fig. 6. Markedly hyperplastic urinary bladder from a rat fed FANFT for 8 weeks showing marked pleomorphism of cells. × 1,000. Inset, the first appearance of pleomorphic microvilli. × 2,000.

Fig. 7. Low-magnification view of urinary bladder from a rat fed FANFT for 12 weeks demonstrating entire surface and advanced papillary lesions (arrows). × 30.

Fig. 8. Low-magnification view of urinary bladder from a rat fed FANFT for 20 weeks demonstrating a single polypoid tumor. × 20.

Fig. 9. Pleomorphic surface cells typical of the predominant cell type in papillary and polypoid lesions from 12-, 14-, and 20-week FANFT-fed animals. × 2,000.

Fig. 10. Rounded microridges which appear to be formed by the fusion of microvilli (arrows). × 6,000.

Fig. 11. Leafy microridges on the surface of markedly hyperplastic bladder. × 10,000.

Fig. 12. Pleomorphic microvilli on the luminal surface of a tumor cell. × 15,000.

Fig. 13. Low-magnification view of cobblestoned surface of a rat fed FANFT for 8 weeks and control diet for 42 weeks. Note elevation of subepithelial blood vessels (arrows). × 200.

Fig. 14. Higher magnification of Fig. 13 demonstrating pleomorphism of cells and the presence of pleomorphic microvilli on luminal cell surfaces. × 4,000.
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