To determine if a population of self-renewing CSCs exist within the cultured human CCRCC, cell line 786-O and clinical specimen of RCC cells were grown in the presence of 10% FBS as MACs and subsequently in SFM containing EGF and bFGF as SFCs, respectively. After 7 d, large tumor spheres with 100 μm diameter appeared (Fig. 1(a)), each composed of 100~200 cells, sphere size reached maximum at 14 d with 200 μm diameter and stop growing with necrosis appears in centre. The cell cycle distributions of SFCs and MACs are shown in Fig. 1(b). In contrast with the recent report\(^1\), compared with MACs, the percentage of proliferating cells in SFCs decreased from 54.20% (±4.2%, \(n=3\)) to 28.28% (±2.1%, \(n=3\)) (\(P<0.01\)); the SFCs in G0/G1 phase increased from 45.78% (±5.5%, \(n=3\)) to 71.70% (±2.9%, \(n=3\)) (\(P<0.01\)). Fig. 1(c) shows that MACs proliferated at a significantly (\(P<0.05\)) greater rate than SFCs. This result is consistent with the observations by Zhong et al.,\(^2\) but is inconsistent with the observations by Yu et al.,\(^3\). The self-renewing and differentiation capacity of 786-O SFCs was assessed by dissociating the spheres into single cells and growing them in routine medium or in SFM containing EGF and βFGF at a clonal density of 1000 cells/ml. When floating spheres of SFCs cells were seeded into serum containing medium, these cells showed spindle-like feature and tight attachment replicating the original cell culture (Fig. 1(d)). In the SFM tumor sub-spheres were evident after 5~7 d (Fig. 1(e)), and the sub-spheres can be passed beyond 60 passages (about 15 months), displaying the self-renewing and proliferative capacity of the 786-O tumor spheres.

![Fig. 1 Properties of SFCs and MACs derived from RCC. (a) The propagation of cell cultured in SFM (200 ×). Scale bar, 100 μm. (b) Cell cycle distribution of 786-O SFCs and MACs. (c) Cell proliferation after enzymatic digestion and re-suspended in the routine medium. SFCs and MACs planted in 96 well plate and density was 500 cells/well, cells proliferation was assayed by MTT. (d) When these spheres were seeded in routine culture medium, they showed a spindle-like structure replicating the primary cell culture. (e) Sub-spheres were continuously generated after SFCs acutase enzymatic digest and reseeded in SFM. Scale bar, 200 μm.](image)

References