Hypoxia in tumors is a key factor in radiotherapy since hypoxic cells are more radioresistant against radiation than oxic cells. To improve outcome in radiotherapy, it is crucial to determine degree and spatial distribution of hypoxia non-invasively, and for this, Positron Emission Tomography (PET) in combination with the tracer Fluoromisonidazole (\[^{18}\text{F}\]FMISO) is a promising technique. This study characterizes the hypoxic conditions of three tumor sublines (AT1, HI and H) of the Dunning R3327 prostate tumor model, which differ in histology, volume doubling time, and progression status, using dynamic \[^{18}\text{F}\]FMISO-PET and histology. Measurements were performed for two tumor volumes (average 0.8±0.5 cm\(^3\) vs 4.4±2.8 cm\(^3\)). In addition, the HI-subline was investigated under different breathing conditions (air vs. 100% of oxygen vs. air). Data were analyzed by the standardized uptake values (SUV) as well as by the kinetic parameters \(k_3\) and \(K_i\) of a two-tissue compartment model. The shapes of the time activity curves (TAC) were used to further characterize the sublines. Quantitative immunohistochemical studies of the hypoxic fraction, perfused vessel density and mean vessel size were performed using pimonidazole, Hoechst 33342 and CD31, respectively. Small tumors did not show a significant SUV (0.64±0.36, 0.55±0.10 and 0.45±0.08, for AT1, HI and H sublines, respectively). In large tumors, the SUVs were 1.33±0.52, 1.12±0.83 and 0.63±0.16 for AT1, HI and H sublines and the corresponding pimonidazole-based hypoxic fractions were 0.62±0.23, 0.54±0.24 and 0.07±0.10, respectively. The AT1- was most and H-tumor was least hypoxic for both methods (\(p<0.05\)). In the breathing experiment, those HI-tumors that were hypoxic under air breathing decreased their SUVs significantly when providing oxygen instead. Only poor or no correlations between PET and histological parameters were found, while different parameters originating from the same method, such as SUV with \(k_3\) and \(K_i\) as well as the hypoxic fraction with pimonidazole mean intensity, correlated better. In summary, both methods, PET and histology, were able to discriminate different hypoxic conditions of tumors, which clearly demonstrates the value of static and dynamic PET measurements to assess hypoxia non-invasively. Further studies are required to better understand the connection between hypoxia-related PET-parameters and the underlying tissue properties on a microscopic scale.