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Cancer Epidemiology in Africa

Until quite recently, knowledge of cancer patterns was based primarily on clinical and pathological case series from the 1950s and 1960s, which were the subject of several reviews that drew together information on the relative frequency of different types of cancer in different areas in order to piece together an overall picture (Clifford, Linsell, and Timms 1968; Cook and Burkitt 1971; Oettlé 1964). Statistics on disease mortality are particularly sparse. Only about 0.25 percent of the population of Sub-Saharan Africa is covered by accurate death registration systems. The countries that have reasonably accurate death registration include islands like Mauritius and the Seychelles, which are unlikely to be representative of the region, and no country on the mainland of Sub-Saharan Africa has data of sufficient quality for the estimation of national mortality rates (Mathers et al. 2005). Hence, reliance has to be placed on indirect measures of mortality and on the few cancer registries that do exist across Africa, now covering roughly 8 percent (Parkin et al. 2003) of this population. An exception to this has been South Africa, which until 1990 had almost complete death notification for whites, mixed race "coloureds," and Asian Indians, comprising about 20 percent of the population. Population group identifiers on death notification forms were removed in 1991 but reintroduced in 1998. National coverage of deaths in South Africa across all populations has now increased to over 90 percent (Dorrington et al. 2001).

Since the 1990s there has been a resurgence of interest in cancer incidence in Africa, and data from cancer registries from Sub-Saharan Africa have been published from West Africa in The Gambia (Bah et al. 2001), Mali (Bayo et al. 1990), Guinea (Koulibaly et al. 1997), and Côte d'Ivoire (Echimane et al. 2000). Data from East Africa are available from cancer registries in Kampala, Uganda (Wabinga et al. 2000), and from southern Africa from the Zimbabwe Cancer Registry in Harare (Chokunonga et al. 2000), and the Malawi Cancer Registry in Blantyre (Banda et al. 2001).

Cancer registration in economically underdeveloped populations, such as all the countries of Sub-Saharan Africa, is a difficult undertaking for a variety of reasons (Parkin et al. 2003). The major challenge is to ensure that all new cases of cancer are identified. Cases can be found only when they come into contact with health services: hospitals, health centers, clinics, and laboratories. When resources are restricted, the proportion of the population with access to such institutions may be limited, and the statistics generated will thus not truly reflect the pattern of cancer. The ease with which the cases can be identified also depends on the extent of medical facilities available and the quality of statistical and record systems already in place (for example, pathology request forms, hospital discharge abstracts, treatment records, and so forth). It is impossible to know, without an extensive population survey, what proportion of those with cancer never come into contact with modern diagnostic or treatment services, instead making use only of traditional healers or receiving no care at all.

In the past, studies have suggested that some sections of the population may have been underrepresented in hospital statistics, particularly older women and young men, both of whom were more likely to return to their rural homes to seek care (Flegg Mitchell 1966). However, currently, this underrepresentation is probably rather rare in contemporary urban Africa. Most cancer patients will, eventually, seek medical assistance, although often at an advanced stage of disease. The situation in rural areas may be quite different, but almost all the present-day cancer registries are located in urban centers. From an epidemiological point of view, one must guess at how well the cancer profile from the urban areas reflects that in the country as a whole, given what is known of urban–rural differences in cancer patterns in other areas of the world.

The International Agency for Research on Cancer (IARC) has published the available data on cancer incidence and other cancer data from a variety of sources (Parkin et al. 2003). Such data have also been used to prepare a set of estimates of incidence and mortality at the national level for the year 2002 (Ferlay et al. 2005). These sources are extensively used in this chapter. We also draw upon the few available series from which it is possible to make some inferences about temporal trends in cancer incidence: the two cancer registries with data available in the 1960s—Kampala (Uganda) and Ibadan (Nigeria)—and the mortality data sets from South Africa referred to earlier.

According to the 2002 estimates of cancer incidence for the Sub-Saharan Africa region, about half a million (530,000) new cases of cancer occurred annually, 251,000 in males and 279,000 in females. Table 20.3 shows the leading cancer types by region (including the northern Africa region) and by sex.

Major Cancer Types in Sub-Saharan Africa, Both Sexes, All Ages *Source:* Adapted from Ferlay et al. 2004.

The top six cancers in males were the following:

- Kaposi's sarcoma (15.9 percent)
- liver (13.3 percent)
- prostate (10.7 percent)
- esophagus (6.0 percent)
- non-Hodgkin's lymphoma (5.8 percent)
- stomach (4.5 percent).

In females, the following were the leading cancers:

- cervix (25.4 percent)
- breast (17.4 percent)
- Kaposi's sarcoma (6.2 percent)
- liver (5.5 percent)
- stomach (3.8 percent)
- non-Hodgkin's lymphoma (3.8 percent).

Each of these cancers is briefly discussed in this chapter. In addition, tobacco-related cancers (especially lung cancer, which currently ranks seventh in males) and HIV-related cancers (cancers aside from Kaposi's sarcoma) are discussed, as these are likely to increase over time as both these epidemics mature.

Cervical Cancer

Cancer of the cervix is the leading cancer in women in Sub-Saharan Africa with an estimated 70,700 new cases occurring in 2002 (the total in the whole continent was 78,900 cases). Estimated rates for eastern and southern Africa of 30 to 60 per 100,000 are higher than those found in the rest of Sub-Saharan Africa (20 to 35 per 100,000), but the reasons for this difference are unclear. In many developed countries, such as the United Kingdom and

Sweden, mortality from cancer of the cervix declined between the early 1900s and the 1960s and then declined further as a result of the introduction of national screening programs (Bergstrom, Sparen, and Adami 1999). However, in Bulawayo between 1963 and 1977 and in Kampala in the 1960s, 1970s, and 1990s, cancer of the cervix has appeared to increase in incidence over time (Skinner et al. 1993; Wabinga et al. 2000). No increases over time were observed in Nigeria and South Africa (Parkin et al. 2003).

It was noted early that cervical cancer has quite marked differences in incidence according to classical demographic variables (social class, marital status, ethnicity, religion). Later, epidemiological studies (mainly case-control studies) showed a consistent association between risk and early age at initiation of sexual activity, increasing number of sexual partners of females or of their sexual partners, and other indicators of sexual behavior. These findings were strongly suggestive of a causative role for a sexually transmitted agent. It is now recognized that certain sexually transmitted oncogenic human papillomaviruses constitute the necessary cause of cervical cancer. However, additional independent risk factors include increasing number of pregnancies, exposure to oral contraceptives, smoking, and specific dietary patterns.

At the onset of the AIDS epidemic, cancer of the cervix was classified as an AIDS-defining cancer by the U.S. Centers for Disease Control and Prevention (CDC 1993). But it is far from clear that HIV infection really increases the risk of invasive cervical cancer. No change in cervical cancer incidence has been demonstrated in some centers like Harare, where HIV/AIDS has been endemic for some time (Chokunonga et al. 1999). In Kampala the increase in cervical cancer incidence began before the advent of AIDS (Wabinga et al. 2000). With respect to cervical intraepithelial neoplasia (CIN), most studies failed to adjust for the fact that, for obvious reasons, women infected by HIV were very often also infected by HPV (with a consequently high risk of CIN). Careful adjustment for such confounding suggests that HIV has an independent effect on risk of CIN but that it is small; there is an interaction between the effects of HIV and HPV, as might be expected, if the role of HIV is indirect, through creation of immune suppression and dysfunction (Mandelblatt et al. 1999).

Case-control and descriptive studies on cancer of the cervix in Africa have shown associations of the disease similar to those observed in Western countries with respect to number of partners, level of education, high parity, and steroid contraceptives; however, genital hygiene, vaginal discharge, alcohol, and male circumcision were also found in certain studies to be important (Parkin et al. 2003). HIV was found to be associated with cervical cancer in case-control and cohort studies in South Africa and Uganda (Mbulaiteye et al., forthcoming; Newton et al. 2001; Sitas et al. 2000) with odds ratios between 1.6 and 2.4; however, such a weak association could easily be due to confounding by sexual activity, and other studies have shown no association (Newton et al. 1995; Sitas et al. 1997; ter Meulen et al. 1992). With regard to HPV, subtypes 16, 18, and 31 appear to be the leading ones, but other sexually transmitted infections causing chronic cervico-vaginal inflammation may increase the risk of cervical cancer.

Before the introduction of screening programs in the 1960s and 1970s, the incidence in most of Europe, North America, and Australia and New Zealand was much as we see it in Africa today: it was 38 per 100,000 in the Second National Cancer Survey of the United States, for example (Dorn and Cutler 1959). National screening programs have been responsible for the further decline in the incidence of cancer of the cervix. Pap test screening, with coverage of over 80 percent of the female population over 35 years of age appears to be the most effective method in reducing the incidence of cervical cancer. For example, if women were offered

screening three times in their lifetime (at about ages 35, 45, and 55) the incidence of cancer of the cervix would be halved (Miller 1992).

Given the complex organization of screening programs, no organized national cervical cancer screening program exists in Africa. Reasons for this include lack of good quality cytology services, difficulty of long-term follow-up in many communities, lack of education, and lack of postal facilities and infrastructure. But many countries in Sub-Saharan Africa do not have the ability to diagnose or treat CIN. In other countries some attention has been given to the value of screening by visual inspection after acetic acid impregnation of the cervix (University of Zimbabwe/JHPIEGO Cervical Cancer Project 1999). The high negative predictive value of this approach suggests that few significant lesions will be missed. If appropriately and safely treated by effective, affordable methods like cryotherapy (Chirenje et al. 2001), then this method may provide a useful alternative to the conventional Pap test, not least in that treatment is provided during the same visit as the screening test, thus dispensing with the requirement to recall women for diagnosis and therapy.

Vaccines against the leading HPV serotypes have now been developed, and programs may be implemented for women before they become sexually active. However, it is unclear how long the protection will last and whether the vaccine will also be effective in reducing the incidence of cancer of the cervix among women who are infected. The ongoing trials are expected to clarify such issues. As men are also carriers of HPV, future studies ought to measure any added effectiveness of vaccination in this group.

Breast Cancer

Breast cancer is the second most common cancer among women in Sub-Saharan Africa, accounting for 16.8 percent of all female cancers. Central, West, and East Africa appear to have lower incidence rates than southern Africa, the latter estimated at 33.4 per 100,000. An estimated total of 48,600 cases occurred in Sub-Saharan Africa in 2002.

Worldwide, risk factors for female breast cancer include menstrual and reproductive factors, high body mass index (BMI), family history of breast cancer, and certain genetic mutations, including BRCA1/2. Other suggested risk factors include, to a much lesser extent, high alcohol consumption, contraceptive use, and the use of certain postmenopausal hormone replacement therapies. Reproductive and hormonal factors appear to be the most important, with risk being increased by early menarche, late menopause, late age at first birth, and low parity (Henderson, Ross, and Bernstein 1988).

Studies in Sub-Saharan Africa have also found reproductive and hormonal factors to be important, reporting increased risk with advanced age at first pregnancy and delivery, low parity, and late age at menarche (Adebamowo and Adekunle 1999; Coogan et al. 1996; Shapiro et al. 2000; Ssali, Gakwaya, and Katangole-Mbidde 1995).

In Sub-Saharan Africa, higher incidence rates and relative frequencies of breast cancer have been reported in association with urban than with rural residence (Oettlé and Higginson 1966; Schonland and Bradshaw 1968), but data are sparse. The incidence of breast cancer is much higher among white women in Africa than among black African women; for example, in Harare between 1993 and 1995, the incidence was 127.7 per 100,000 in whites and 20.4 in blacks (Chokunonga et al. 2000). These differences may be a reflection of the distribution of lifestyle factors thought to be important in the development of breast cancer, for example, low parity and high body mass.

Breast cancer risk has been associated with socioeconomic status, with women of higher social class (as measured by education, income, housing, and so forth) having a higher risk (Kogevinas et al. 1997). Once again, such differences are most likely a reflection of different prevalences of risk factors among social classes (for example, parity, age at menstruation and menopause, height, weight, alcohol consumption).

The effect of oral contraceptive hormones on the risk of breast cancer has been the subject of much research. There appears to be a small but detectable risk in women currently using oral contraceptives, but this diminishes when contraception ceases, and after 10 years, none of the excess risk remains (Reeves 1996). A case-control study in South Africa found that combined oral contraceptives may result in a small increase in risk, confined to women below the age of 25 years, but that injectable progesterone contraceptives did not increase risk (Shapiro et al. 2000).

Dietary fat appears to be correlated with the risk of breast cancer in interpopulation studies (Prentice and Sheppard 1990), but the association has been difficult to confirm in studies of individuals (Hunter et al. 1996). However, obesity in postmenopausal women has been identified as a risk factor in Europe (Bergstrom et al. 2001) as well as in Sub-Saharan Africa (Adebamowo and Adekunle 1999; Walker et al. 1989). Although traditional diets in Africa are typically low in animal products, especially fat, and high in fiber (Labadarios et al. 1996; Manning et al. 1971), this pattern is being modified by urbanization and Westernization of lifestyles, which may lead to an increase in breast cancer incidence in African populations. A case-control study in Cape Town did not find a protective effect of breastfeeding on breast cancer (Coogan et al. 1999). However, in a meta-analysis of 47 studies from 30 countries breastfeeding appears to be protective; based on a reanalysis of about 50,302 cases and 96,973 controls, two-thirds of the difference in rates between developed and developing countries were estimated to be attributed to breastfeeding (International Collaboration on HIV and Cancer 2002).

At least part of the familial risk of breast cancer is mediated through the major susceptibility genes BRCA1 and BRCA2 (about 2 percent of breast cancer cases in Europe). Very little is known of the prevalence of these mutations in African populations, although family history of breast cancer is also a risk factor in this setting (Rosenberg et al. 2002).

About 1 percent of all breast cancer cases occur in men, with the male-to-female ratio being higher in black and African populations than among white populations (Parkin et al. 2003; Sasco, Lowels, and Pasker de Jong 1993).

A review of the literature indicates a deficit of studies on breast cancer risk in Sub-Saharan Africa, and further research could be beneficial. As certain groups become more Westernized and urbanized, with associated changes in diet, later childbirth, and reduced parity and periods of breast-feeding, breast cancer incidence may increase. Public health campaigns should encourage breastfeeding unless there are good reasons not to (for example, HIV-infected mothers where milk powder and sterile water are freely available). There is no organized mammography screening program in Sub-Saharan Africa.

Kaposi’s sarcoma

Prior to the HIV/AIDS era, Kaposi's sarcoma was a rare cancer in Western countries, seen mainly among immigrants from the Mediterranean littoral and African regions and in immunosuppressed transplant recipients. Meanwhile, in Africa, the incidence of Kaposi's sarcoma varied 100-fold, being most common in central and eastern Africa and rare in northern and southern Africa (IARC 1996; Oettlé 1962); in certain parts of central and

eastern Africa, Kaposi's sarcoma was as common as cancer of the colon was in the West (Cook-Mozaffari et al. 1998). There appears to be some geographical association with the prevalence of human herpes virus-8, now regarded as a necessary cause for the development of Kaposi's sarcoma (Dukers and Rezza 2003). The incidence of Kaposi's sarcoma has increased over 1,000-fold in populations at high risk of HIV in some Western countries (Biggar et al. 1984; Rabkin, Biggar, and Horm 1991), but in the rest of the population the tumor still remains relatively rare (Grulich, Beral, and Swerdlow 1992; Rabkin, Biggar, and Horm 1991). In Africa, since the 1980s, areas like Malawi, Swaziland, Uganda, and Zimbabwe, where Kaposi's sarcoma was relatively common before the era of AIDS, the incidence of Kaposi's sarcoma has increased about 20-fold, such that it is now the leading cancer in men and the second leading cancer in women. In these cancer registries, overall age-standardized rates have increased by about 15 percent, mainly as a result of HIV-associated Kaposi's sarcoma (for example, Bassett et al. 1995; Wabinga et al. 1993; Wabinga et al. 2000).

According to the most recent estimates, 40,000 cases of Kaposi's sarcoma in males and 17,200 cases in females were estimated for 2002 for Sub-Saharan Africa; only 200 male and 65 female cases were estimated to occur in northern Africa. The region most affected is central Africa (age-standardized rates in males of 30 per 100,000) followed by eastern, southern, and lastly western Africa, in line with the background prevalence of HIV in each of these regions. With regard to the effect of HIV infection, three case-control studies from Africa showed increased risks of 30 to 50 in association with HIV, and these risks rise to 1,600 in HIV-positive individuals with high HHV8 antibody titers (Newton et al. 2002; Sitas et al. 1997; Sitas et al. 1999; Sitas et al. 2000). HHV8 in adults is associated with increasing age, low educational standard, and increasing numbers of sexual partners (Sitas et al. 1999). Antiretroviral therapy for treating HIV in adults has caused a decline in the incidence of Kaposi's sarcoma in Western countries (International Collaboration on HIV and Cancer 2000). HHV8 in children appears to be associated with infected mothers (Bourboulia et al. 1998). In countries with a high prevalence of HIV, Kaposi's sarcoma is now the leading cancer in children, causing almost a doubling in the childhood cancer incidence (Chokunonga et al. 1999; Wabinga et al. 1993). Antiretroviral drugs have now become more available in Botswana and recently in South Africa. If their use becomes widespread, then a decline in the incidence of Kaposi's sarcoma would be expected; however, it is unclear whether antiretrovirals (for example, zidovudine [AZT] or nevirapine) issued to mothers during delivery, which proved effective in reducing mother-child transmission of HIV, would cause a decline in Kaposi's sarcoma in children.

### Stomach Cancer

A total of 13,800 cases of stomach cancer in males and 10,700 in females was estimated in Sub-Saharan Africa in 2002. Age-standardized incidence rates in males varied, per 100,000, from 3.4 in western Africa to 7.4 in eastern, 8.2 in southern, and 13.4 in central Africa. In western Africa, where the incidence of stomach cancer is the lowest, the male-to-female ratio is 0.9 to 1; however, there is a male predominance in all other areas (table 20.3). Despite the generally low incidence rate in Africa, some populations have a particularly high incidence rate. Clusters of high incidence exist among the South African mixed race, or coloured, population of 98 per 100,000. A high incidence rate is also reported in the Great Lakes region that includes Burundi, Kivu Province of the Democratic Republic of Congo, Rwanda, northwestern Tanzania, and southwestern Uganda. In Rwanda the age-standard incidence rate was found to be 13 per 100,000 males and 15 per 100,000 females (Newton et al. 1996). In western Uganda, stomach cancer was the second most common cancer, accounting for 12

percent of all male cancers and 6 percent of all female cancers (Wabinga et al. 2000). Bamako in Mali was another area with a high incidence rate: 18.5 per 100,000 males and 15 per 100,000 females (Bayo et al. 1990).

There is evidence of a slight but not significant increase in the incidence of stomach cancer over time in Kampala (Wabinga et al. 2000). In Kivu Province of the Democratic Republic of Congo, the incidence rates of stomach cancer among males and females were 9 and 15 per 100,000, respectively, in 1956–60, but this dropped to 6 and 4.5 per 100,000 in 1983–86 (Bourdeaux et al. 1988; Clemmensen, Maisin, and Gigase 1962). However, in rural Kenya reported incidence increased as a result of an endoscope acquired by the main hospital there (McFarlane et al. 2001). Trend data from the rest of Africa are incomplete or inconsistent; however, in South Africa, between 1948 and 1964 no real change in the relative frequency of stomach cancer was observed over time in one of the country's largest hospitals serving the predominantly black population of Soweto, Johannesburg (Robertson 1969), nor was a change observed in pathology-based cancer national registrations between 1986 and 1995 (Sitas, Madhoo, and Wessie 1998).

*Helicobacter pylori* infection is now recognized as an important risk factor for cancer of the stomach (IARC 1994); however, smoking and diets low in fruit and vegetables and vitamin C, and high in salts appear to play an important role. Many studies have shown the prevalence of *H. pylori* in Africa to be about 80 percent and that infection is acquired at a younger age than in Western countries (for example, Sathar et al. 1994). Chronic atrophic gastritis and intestinal metaplasia of the stomach are two key lesions in the natural history of stomach cancer. Very few studies in Sub-Saharan Africa have measured the association between gastric mucosal pathology and *H. pylori*. In summary, even in a continent where the prevalence of *H. pylori* is high, differences exist in the prevalence of *H. pylori* between those with a normal mucosa (0–33 percent) and those with gastritis of any kind. Needless to say, the prevalence of gastritis (mild or moderate) is high, but the prevalence of severe or chronic atrophic gastritis or intestinal metaplasia is low (Parkin et al. 2003). Two case-control studies from Africa show an association between *H. pylori* and stomach cancer, but the relative risks are low, probably because the mucosa of patients with gastric cancer is unfavorable to the survival of *H. pylori* (Jaskiewicz et al. 1989; Louw et al. 2001). CagA positive strains, usually associated with more severe gastric pathology and outcomes, are the predominant strains in Africa (Ally et al. 1998), but their role in gastric carcinogenesis is unclear. Certain vacA genotypes appear to be more common in patients with gastric cancer (Kidd et al. 1999) and seem to be independent risk factors for the disease; however, no studies have been done in Sub-Saharan Africa on the relation between stomach cancer, host susceptibility (in relation to inflammatory cytokines), and the other risk factors known to be associated with stomach cancer (for example, diet, salt, smoking, and pickled foods) (see, for example, Coggon et al. 1989). There are many places in Africa where food is salted or pickled to aid preservation, but the relative importance of these risk factors in local settings is unknown.

Liver Cancer

Early observations in Africa have always noted the high occurrence of liver cancer (for example, Oettlé 1964), and it is still one of the leading cancer types in men and women, although the relative frequency has been reduced in consequence of the large increase in the number of cases of Kaposi's sarcoma resulting from the epidemic of HIV/AIDS. Liver cancer is now the second leading cancer in men in Sub-Saharan Africa and the fourth leading cancer in women. There were an estimated total of 33,500 cases in males and 15,500 cases in females in 2002. Areas of high liver cancer incidence (mainly hepatocellular cancers) include countries like The Gambia, Guinea, and Senegal in West Africa, where liver cancers

comprise a quarter or more of all cancer cases, with incidence rates ranging from 30 to 50 per 100,000 in men and 12 to 20 per 100,000 in women. Similarly, in central Africa, liver cancer is the leading cancer in Rwanda and in the Republic of Congo (Brazzaville); the estimated rate is 15.4 per 100,000 for men and 8.9 per 100,000 for women. Mozambique is reported to have high incidence rates, although the only data are old (Prates and Torres 1965).

Few places in Sub-Saharan Africa have information on cancer trends over time. In Ibadan, Nigeria, between 1960–69 and 1998–99, there appears to be no change in incidence, whereas in Kampala, Uganda, between the 1960s and the 1990s there appears to be a decline of liver cancer in men but not in women. However, a decline was noted in liver cancer incidence between the 1970s and the 1980s among Mozambican miners working in South Africa (Harington, Bradshaw, and McGlashan 1983).

Chronic carriage of HBV or hepatitis C (HCV), causing cirrhosis, or chronic hepatitis is the leading risk factor for liver cancer. The prevalence of HCV in Sub-Saharan Africa varies between 6.9 percent in central Africa to 0.1 percent in southern Africa. HCV transmission is probably via blood transfusion, unsterile medical and dental procedures, and traditional practices, such as scarification; sexual transmission is thought to be rare (Madhava, Burgess, and Drucker 2002).

Persistence of the HBV surface antigen (HbsAg) in blood is an indicator of chronic carriage of HBV infection. The risk of liver cancer in persons with chronic HBV infection, as indicated by the detection of HbsAg in serum, ranges from 6- to 20-fold in different studies, and it is estimated that about two-thirds of liver cancer in Africa is attributed to HBV (Pisani et al. 1997). Prevalence rates in Africa are over 10 percent in central, western, and eastern Africa and between 5 and 10 percent in southern Africa (Parkin et al. 2003).

There are relatively few African studies on the risk of HCV infection on the development of liver cancer. Those that have been conducted give relative risks ranging from 1.1 to 62 (Parkin et al. 2003). One study (Kirk et al. 2004) observed that, as has been found elsewhere, the risk of chronic infection by HCV and HBV is additive, suggesting common mechanisms of carcinogenesis.

Aflatoxin B1 (AFB1) is produced by molds of *Aspergillus* sp. that are common contaminants of poorly stored grains. AFB1 is a known liver carcinogen of animals and humans (IARC 1993, 2002). In Sub-Saharan Africa, high levels of AFB1 contamination are found in groundnuts and, to a lesser extent, corn. Contamination of groundnuts by AFB1 is quite widespread and frequently exceeds thresholds permitted in exports to most developed countries. Several geographical studies have demonstrated correlations between AFB1 levels and the incidence of hepatocellular cancer (see Parkin et al. 2003).

Iron overload, derived from food and drink preparation in iron vessels, is a common condition in rural Africa, and there have been several observations that elevated serum ferritin levels are associated with liver cancer. In one small case-control study in South Africa (Mandishona et al. 1998), liver cancer cases had higher iron overload levels than controls, corresponding to an odds ratio of 10.6 to 4.1 (depending on the control group used).

Smoking, oral contraception, and alcohol consumption (IARC 2004, 1999, and 1988, respectively) were also found to be important risk factors for liver cancer. This association, however, has not been extensively examined in Africa.

Early vaccine trials against HBV suggest that 70 to 75 percent of chronic infections could be prevented. A randomized trial to measure the effectiveness of HBV vaccination in the

prevention of liver cancer is under way in The Gambia, but it will take many years before results are available. In Taiwan, however, children born after the introduction of mass vaccination had a fourfold lower incidence than those born before its introduction (Chang et al. 1997). According to the WHO Web site, by 2002, about a dozen countries in Sub-Saharan Africa had introduced hepatitis B vaccine into their infant immunization system (http://www.who. int/vaccines-surveillance/graphics/htmls/HepBvaccine UseMar02.htm).

Aflatoxin consumption could be reduced by improved education of individuals and farmers by, for example, agricultural extension officers. A trial in western Africa has shown that improved post-harvest storage of groundnuts can significantly reduce aflatoxin exposure in rural populations (Turner et al. 2005). The public could be educated to avoid contaminated peanuts sold by vendors (Wild and Hall 2000). Companies manufacturing peanut butter could be better controlled by accepting peanuts only from certified farmers and by the testing of their products by independent regulatory authorities.

Prostate Cancer

For the year 2002, a total of 26,800 cases of prostate cancer were estimated, comprising 10.6 percent of cancers of men in Sub-Saharan Africa (Ferlay et al. 2005). The relatively high incidence (and mortality) recorded in African populations is reflected in populations of African descent elsewhere. Thus, within the United States, the black population has the highest incidence (and mortality) rates, some 72 percent higher than whites. Southern Africa appears to have the highest rates (40.5 per 100,000). Rates of histologically diagnosed prostate cancer in South Africa are 40.1 per 100,000 in whites versus 14 per 100,000 in blacks, although for blacks, access to diagnostic facilities has been limited (Parkin et al. 2003). In Zimbabwe (defined as being part of eastern Africa), rates for whites and blacks were 70 versus 25 per 100,000 (Parkin et al. 2003). Central Africa follows with rates of 24.5 per 100,000. Surprisingly, in West Africa, where the majority of African-American men originated, the incidence rate of prostate cancer was estimated as 19.3 per 100,000 in 2002, compared with about 125 per 100,000 in the United States (Ferlay et al. 2005). High rates are observed in other places with populations that are descended from West Africa (for example, the Bahamas, Barbados, Trinidad).

Histology of the prostate in elderly men often reveals latent malignant cells, and clearly, advances in diagnostic and screening methods can cause artificial increases in reporting. This is illustrated by a fourfold increase in the incidence of histologically verified prostate cancer among whites in South Africa (most whites were covered by private health insurance) compared with no change in incidence in blacks between 1986 and 1995 (Sitas, Madhoo, and Wessie 1998). Notably, in Cape Town in the 1950s prostate cancer appeared to be more common in blacks than in whites (Muir-Grieve 1960). Increases over time have also been noted in Kampala and in Ibadan, but it is unclear how much of these increases represents a greater risk and how much can be attributed to increased awareness or a greater readiness to perform prostatectomy for urinary symptoms in elderly men (Parkin et al. 2003).

The consumption of fat and red meat has been implicated as a risk factor for prostate cancer in studies in developed countries, even though adjustment for total caloric intake was not always done. Associations with vegetable consumption have been inconclusive. Associations with anthropometric measures or a link with obesity have been inconclusive, and so have associations with numbers of sexual partners and history of sexually transmitted diseases, or STDs (Hayes et al. 2000; Key 1995; Kolonel 1996). In one case-control study from South Africa, prostate cancer was associated with high intake of fat, meat, and eggs; eating out of the house; and a low consumption of vegetables (Walker et al. 1992).

Sex hormones, modulated by polymorphisms on the long arm of chromosome X, play an important role in the development of prostate cancer (for example, Ross et al. 1998; Shibata and Whittemore 1997). Polymorphisms on the androgen receptor gene may vary by ethnic group and may provide some explanation for the geographic variation observed. However, no studies have been done on interethnic variations in androgen receptor polymorphisms in Africa.

Non-Hodgkins Lymphoma

The non-Hodgkin's lymphomas are composed of an extremely heterogeneous group of lymphoproliferative malignancies displaying distinct behavioral, prognostic, and epidemiological characteristics. Advances in molecular biology, genetics, and immunology have resulted in extensive changes in the classification of lymphoid tumors in the last few decades. The WHO classifies tumors according to cell lineage defined by immunophenotype (Jaffe et al. 2001). Three broad categories are now recognized: B-cell neoplasms, T/NK-cell neoplasms, and Hodgkin's lymphoma. Lymphocytic leukemias fall within the B-cell neoplasm group.

A total of 14,500 cases in males (5.8 percent of all cancers) and 10,600 cases in females (3.8 percent of all female cancers) were estimated for 2002 in Sub-Saharan Africa. In most African populations non-Hodgkin's lymphoma is relatively rare, but the relative frequency is above the world average in North and Sub-Saharan Africa because of the high incidence of Burkitt's lymphoma in children in the tropical zone of Africa. As in Western countries, most non-Hodgkin's lymphomas in Africa are of B-cell type. In adults, clinical series show an excess of high-grade lymphomas and a deficit of nodular lymphomas.

Human T-cell lymphotrophic viruses (for example, HTLV-I) are common in tropical Africa (IARC 1996) and are a cause of T-cell lymphomas; however, the incidence of these in Africa is low. Although Epstein-Barr virus DNA may be found in a small proportion of lymphomas, its role in causing non-Hodgkin's lymphomas is unclear (IARC 1997). HCV infection has been implicated in B-cell non-Hodgkin's lymphomas in some studies; the postulated mechanism being through the stimulation of polyclonal proliferation of B cells (reviewed by Parkin et al. 2003). HIV infection has been associated with 60-fold increased risks of developing non-Hodgkin's lymphomas in Western countries (for example, Beral et al. 1991); approximately 5 to 10 percent of HIV-infected persons will develop a lymphoma, and non-Hodgkin's lymphoma is the AIDS-defining illness in about 3 percent of HIV-infected patients (Remick 1995). In Africa, however, the association between HIV and non-Hodgkin's lymphoma has been in the region of 2.3 to 12.3 (Mbulaiteye et al., forthcoming; Newton et al. 2001; Parkin et al. 2003; Sitas et al. 1997; Sitas et al. 2000). The reason for the discrepancy in the association between HIV and non-Hodgkin's lymphoma between developed countries and Africa is unclear. Non-Hodgkin's lymphomas were increasing in incidence in Western populations before the advent of HIV but have increased dramatically in high-risk groups affected by HIV (see, for example, Schultz, Boshoff, and Weiss 1996). In Harare, Zimbabwe (Chokunonga et al. 1999), and in Kampala, Uganda, there is now evidence of an increase in incidence between earlier cancer registration periods and periods in the 1990s (Parkin et al. 1999; Parkin et al. 2003).

Burkitt's lymphoma affects mainly children between the ages of five and nine. The jaw is affected 50 to 60 percent of the time. Burkitt's lymphoma shows a peculiar geographic distribution and has been reviewed by others (for example, Burkitt 1969; Williams et al. 1978; Wright 1973). It accounts for about a quarter to a half of childhood cancers in the eastern and central parts of Africa and in tropical West Africa, and less frequently in other

places. Burkitt identified a striking distribution 15 degrees north and south of the equator, with a southern tail into Mozambique. But even within this area Burkitt's lymphoma was rarer in higher altitudes. The areas where it was most common were typified by rainfalls over 50 centimeters per year and an average of the coolest month of greater than 15.6°C, which seem associated with the distribution of malaria endemicity (Burkitt 1969; O'Conor 1970). Low socioeconomic status, family clustering, and proximity to the plant species *Euphorbia tirucalli* have been suggested as important factors in the etiology of Burkitt's lymphoma; however, the leading agent has been infection with Epstein-Barr virus (IARC 1997).

In a follow-up study of 42,000 children, those who developed Burkitt's lymphoma had higher titers of antiviral capsid antigen than in matched controls (de Thé et al. 1978; Geser et al. 1982). The link with malaria appears to be a result of the loss of cytotoxic T-cell control due to dysfunction of a subset of CD4 cells responsible for the induction of suppressor-cytotoxic CD8 cells. This may result in uncontrolled proliferation of B cells containing the Epstein-Barr virus and resultant malignant transformation (for example, Pagano et al. 1992; Whittle et al. 1990). In a five-year period of malaria suppression (when chloroquine was issued to children under 10), Burkitt's lymphoma appeared to decline in incidence. Incidence returned to the original level after the five-year program was completed (Geser, Brubaker, and Draper 1989). Burkitt's lymphoma is much rarer in adults, although Burkitt-like (or high-grade Burkitt-like) lymphomas appear to be occurring with increased frequency as a result of HIV (Sitas et al. 2000).

A prevention program for non-Hodgkin's lymphomas can be carried out only after the taxonomy and causes are further elucidated. It appears that antimalarial programs may have a significant impact on Burkitt's lymphoma in children, and as in Western countries, widespread antiretroviral therapy of HIV-positive individuals would cause a decline in the incidence of non-Hodgkin's lymphoma.

Cancer of the Oesophagus

In 2002 a total of 15,150 cases of cancer of the esophagus were estimated to occur in males in Sub-Saharan Africa and 7,200 cases in females. Cancer of the esophagus shows a remarkable geographic distribution, being one of the leading cancers in southern and East Africa (average incidence in males about 19 per 100,000) but rare in West Africa (1 to 2 cases per 100,000). Certain areas of high risk have been reported from Kenya and the former Transkei homeland in the Eastern Cape Province of South Africa, where incidence rates as high as 76.6 per 100,000 in males and 36.5 per 100,000 in females were reported between 1991 and 1995 (Somdyala et al. 2003). Several studies between the 1950s and the 1990s in South Africa, Uganda, and Zimbabwe have demonstrated that cancer of the esophagus has increased in incidence. But the latest available data from cancer registries in these countries show a declining trend in esophageal cancer incidence, particularly in males after 1990 (Parkin et al. 2003; Somdyala et al. 2003).

Tobacco and alcohol consumption, known risk factors for the development of esophageal cancer in many countries, have also been documented as important in Africa in studies conducted from the 1980s onward; earlier studies found no such association, probably because of the low alcohol concentration of noncommercial drinks. The net effect of increasing commercial alcohol consumption, combined with increases in some places of tobacco consumption, on esophageal cancer trends is to date unclear. There is no consistent evidence of an effect of homemade brews and esophageal cancer risk in Africa.

Esophageal cancer also appears to occur in areas of extreme poverty and poor nutritional status. The high incidence of esophageal cancer in the Transkei region of the Eastern Cape Province has been associated with the monotonous consumption of corn, which contains low levels of niacin, riboflavin, vitamin C, zinc, calcium, and magnesium (Van Rensburg 1981) and is sometimes contaminated with fungal toxins produced by *Fusarium* spp. Certain studies in this region have shown a geographical association with the presence of *Fusarium moliniforme,* a common fungal contaminant of poorly stored corn. Other risk factors reported in the Transkei include infections with *Candida albicans* and the consumption of a green, leafy plant weed, *Solanum nigrum* (Sammon 1992).

Tobacoo Relate Cancer

Tobacco smoking is by far the most important cause of lung cancer. The evidence has been reviewed many times (IARC 1986, 2004). In 1985 it was estimated that about 76 percent of all lung cancer worldwide (84 percent of cases in men and 46 percent in women) could be attributed to tobacco smoking (Parkin et al. 1994). However, in Africa, because smoking is a relatively recent habit in most areas, the proportion of tobacco-attributed lung cancers is low.

Only where the smoking habit has been established in a significant percentage of the population for a prolonged period of time is the proportion of tobacco-attributable cancers also significant—85 percent of cases in males in certain southern African populations and 68 percent in northern Africa, for example (Parkin and Sasco 1993).

Because of the lower incidence of lung cancer in Africa (and the low prevalence of tobacco consumption in most places in Africa) there is a widespread misconception that the hazards of tobacco are only relevant in developed countries. However, it appears that tobacco consumption, particularly of manufactured cigarettes, is increasing in Africa. Figure 20.3 shows the distribution of per capita consumption of cigarettes in Africa in countries where data exist. It is notable that aside from southern and northern Africa, consumption is low. Typical per capita consumption in the United States, for example, is 2,255 cigarettes, and in China, 1,791, per year. In a WHO survey it was found that between two decades, 1970–72 and 1990–92, 15 countries in Africa increased their consumption of cigarettes, 6 decreased, and 5 remained unchanged (WHO 1997). Data were unavailable for the rest of Africa. Adult smoking rates also vary significantly; prevalence in Africa among men varies from 10 to 50 percent, and among women from 1 to 10 percent (WHO 1997). An exception may be the mixed-race population of South Africa, where there has been a high prevalence (currently 40 to 50 percent) of smoking among women, and, indeed, lung cancer rates (and rates for other tobacco-associated cancers such as oral and esophageal cancers) are higher in southern Africa than the rest of Africa.

Evidence of the recent effect of the tobacco epidemic in Africa comes from the Northern Province (mainly rural) of South Africa (Mzileni et al. 1999) and Soweto (urban South Africa) (Pacella-Norman et al. 2002). In the Northern Province, the relative risk that males would develop lung cancer if they smoked 15 cigarettes or more per day was 13, but in Soweto the relative risk for smoking the same number of cigarettes was 20.7. The latter relative risk is comparable to that observed in some developed countries.

In a study from South Africa, which uses the death registration system to ask the next of kin about the smoking status of the deceased, 61 percent of male and 48 percent of female deaths due to lung cancer were found to be attributed to smoking (compared with 80 to 90 percent in men and 30 to 70 percent in women in Western countries (Parkin and Sasco 1993). It was estimated that in all about 22,000 adult deaths (8 percent of total deaths compared with 15

percent of deaths in Western countries; Peto et al. 2004) were attributed to tobacco (Sitas et al. 2004). Surprisingly, more deaths from chronic obstructive pulmonary disease and tuberculosis would be expected from tobacco than deaths from lung cancer. The reason for the lower proportions of lung cancers attributed to smoking is that some of such cancers can be attributed to occupational exposures, environmental tobacco smoke, air pollution, and radon gas exposure (Parkin and Sasco 1993). Other tobacco-attributed cancers studied in Africa include those of the bladder, cervix, larynx, and esophagus and oral cancers (summarized in chapters in Parkin et al. 2003).

In Nigeria,

Our study shows that the commonest cancers in Nigeria in 2009 to 2010 were breast and cervical cancer among women and prostate cancer among men. We found significant increase in the incidence of breast cancer compared to historical records while the incidence of cervical cancer was relatively stable. There was very little disparity in the cancer incidence reported by registries in the northern and southern parts of the country regardless of differences in ethnicity and level of urbanization.

Our data suggests that the incidence of breast cancer in Nigeria has risen significantly. The age standardized incidence rates for breast cancer in the period 1960–1969 was 13.7 per 100 000 and it rose to 24.7 per 100 000 by 1998–1999 – more or less a doubling of incidence over 4 decades or approximately 25% increase in incidence per decade. [11] With incidence in 2009 to 2010 at 54.3 per 100 000, this represents a 100% increase in the last decade. This is supported by the literature showing rise in breast cancer incidence rates in SSA[18] In our results, the incidence rate of breast cancer in Nigeria in 2010 was higher than the GLOBOCAN 2008 estimate of 38.7 per 100 000 [2], although the GLOBOCAN estimate is for the whole country and for a different time period. The increase in cancer incidence in women may be both apparent and real. Some of the increase noted may result from improved diagnosis, better case finding and improved access to care. Despite this, some of the increase in incidence may be real due to increasing prevalence of risk factors for these cancers in populations that hitherto had low incidence.[18]

Both registries in our study reported similar proportion of cases in males and in females and women had 2 times more cancers than men. The most common cancers in Nigeria were breast, cervix and prostate cancers. Possible explanations for the higher proportion of cancers occurring in women include differences in incidence pattern of cancers that occur commonly in both sexes compared to the age structure of the population. Other reasons include the relative ease of diagnosis and more specific symptomatology of common female cancers compared with those in men (prostate and liver), more frequent contact with the health care system by women due to uptake of maternal/child health care services, greater population awareness of breast and cervical cancers, better health-seeking behavior by women compared to men.[17]

The basis of diagnosis is a measure of validity in a cancer registry. Accuracy of diagnosis is notably higher if it is more frequently based on histological verification.[21] When the basis of diagnosis with histological verification is less than 80%, this may mean that there is poor validity[22]. International recommendations support a morphological basis of diagnosis of between 80 – 90%[22]. In contrast, when the basis of diagnosis includes morphological verification of greater than 90%, this may mean that there is a lack of completeness as the registry is utilizing mostly pathology laboratories at the expense of other notification sources[21]. Basis of diagnosis using histology of the primary tumor in ABCR was about 70% and approximately 45% in IBCR. Cytology was used in the diagnosis of 24% of the

cases in ABCR. Clinical diagnosis was the second most common basis of diagnosis of cancer cases in IBCR (41%). This percentage of clinical diagnosis is quite high and may be related to high prevalence of advanced stage cancers in the hospital base of the IBCR. It has been reported in the literature that high numbers of patients diagnosed clinically is an indicator of late diagnosis or lack of resources in the country [22] and advanced stage at presentation is a common finding in Nigerian cancer patients.[23; 24]

Cervical cancer is the most common cancer among women in most of sub-Saharan Africa [25; 26]. However in Nigeria, it is the second most common after breast cancer.[2] Our findings are also somewhat similar to the 2008 GLOBOCAN estimates for the whole country of 32.9 per 100 000 for the period 1998 to 2002.[2] This suggests that the incidence of cervical cancer has remained largely stable over time. This seemingly stable incidence of cervical cancer over time could possibly be due to poor screening coverage in Nigeria.

Prostate cancer was the overall most common cancer in both registries. However, there was no significant difference in the incidence rate of prostate cancer between both registries. (Table 3) This was surprising because we expected that Abuja would have a significantly higher incidence rate of prostate cancer due to difference in socio-economic status of the populations covered by the Ibadan and Abuja as Ibadan residents have a lower socio-economic profile than Abuja. In the literature, the biological, socioeconomic and socio-cultural determinants of prostate cancer risk including low socio-economic status are highlighted among a population of African Americans in the US as compared with a Caucasian population.[27]

Kaposi sarcoma incidence in men from our study is low compared to Eastern Africa where the ASRs in men in Harare Zimbabwe were 47.2 per 100 000 and 39.3 per 100 000 in Kampala Uganda in 2001.[28] However our findings are similar to findings from other west African countries such as Gambia and Cote d'Ivoire who enjoy relatively low incidence of KS.[28] The total number of KS cases was higher in ABCR compared to IBCR despite the smaller population at risk in Abuja. We speculate that this difference is probably due to the higher prevalence of HIV in Abuja (8.4%) compared to Ibadan (3.9%). [29] However, the cancer registries in this report do not routinely collect data on HIV status, and so the proportion of cases associated with HIV infection could not be ascertained. Findings of higher KS incidence in HIV positive populations, has been reported from Uganda and Zimbabwe.[6; 8] However, with increasing use of highly active antiretroviral therapy, the incidence of Kaposi sarcoma is expected to decrease.[30]

Our study is limited by the fact that the ABCR is a relatively new PBCR and data reported here is for the first 2 years of the registry's existence. Underreporting could occur due to lack of access to treatment and inclusion of only microscopically verified cases in the registry database. Also it is possible that older individuals for cultural reasons may not want to look for care and so may be missed out in the registration process. However, underreporting though possible was unlikely to be high in our data given that somewhat similar findings were obtained from the ABCR compared with the older, renowned IBCR.

Osteosarcoma

Osteosarcoma is a relatively uncommon cancer although it is the most common primary malignancy to arise from bone. While incidence is low, osteosarcoma predominately affects adolescents and young adults, and if untreated it is fatal. Despite modern treatment protocols that combine chemotherapy, surgery, and sometimes radiotherapy, the 5- year survival rate for patients diagnosed with osteosarcoma remains at 60%–70% [1]. Current treatments for

osteosar- coma are associated with significant morbidity, and a period of rehabilitation may be required following surgery for osteosarcoma. Hence, there is a real need to optimise current treatment strategies and to develop novel approaches for treating osteosarcoma. Traditionally, our understanding of osteosarcoma has been largely anatomical. Osteosarcoma arises most com- monly in the metaphyseal region of long bones, within the medullary cavity, and penetrates the cortex of the bone to involve the surrounding soft tissues. A pseudocapsule forms around the penetrating tumour [2]. Histologically, osteosar- coma is characterised as a highly cellular tumour composed

of pleomorphic spindle-shaped cells capable of producing an osteoid matrix. Current standards for staging and surgical resection rely on this anatomical knowledge [3]. However, recent developments in molecular biology have provided insight into the molecular pathogenesis of osteosarcoma. Through the identification of tumour pathways and specific mediators of osteosarcoma progression, novel approaches for targeting osteosarcoma are being developed. This paper will review our current understanding of the molecular pathogenesis of osteosarcoma.

## 2. Pathogenesis

2.1. Bone Growth and Tumorigenesis. Osteosarcoma has a predilection for developing in rapidly growing bone. A number of studies have established a correlation between the rapid bone growth experienced during puberty and osteosarcoma development [4, 5]. Fifty-six percent of all osteosarcomas present around the knee [2]. The epiphyseal growth plates of the distal femur and proximal tibia are responsible for a great deal of the increase in height that occurs during puberty. Additionally, the peak age of osteosarcoma development is slightly earlier for females, an observation that may be explained by the relatively earlier growth spurt experienced by girls [6]. There is a male:female ratio of 1.5:1 for osteosarcoma, and patients affected by the disease are taller compared to the normal population of the same age group [7]. Patients affected by Paget's disease, a disorder characterised by both excessive bone formation and breakdown,alsohaveahigherincidenceofosteosarcoma[2].

2.2. Environmental Factors. Physical, chemical, and biologi- cal agents have been suggested as carcinogens for osteosar- coma. Among these, the role of ultraviolet and ionising radiation is the best established. The initial pathogenic link between radiation exposure and osteosarcoma was noted in female radium dial workers who applied radium to watch faces to make them luminescent [8]. However, radiation exposure is implicated in only 2% of cases of osteosarcoma [9] and is not thought to play a major role in paediatric disease. An interval of 10–20 years between exposure and osteosarcoma formation has been observed [10]. When radiotherapy is used in children as a treatment agent for a solid tumour, 5.4% develop a secondary neoplasm, and 25% of these are sarcomas [11]. The chemical agents linked to osteosarcoma forma- tion include methylcholanthrene and chromium salts [12], beryllium oxide [13], zinc beryllium silicate [14], asbestos, and aniline dyes [15]. Previously, a viral origin had been suggested for osteosarcoma. This stemmed from the detec- tion of simian virus 40 (SV40) in osteosarcoma cells. However, the presence of SV40 in these cells was later concluded to be the result of presence of SV40 viral units as contamination in the polio- virus vaccine that these patients had received [16, 17]. Studies evaluating the role of SV40 in the pathogenesis of mesothelioma have suggested that detection of SV40 in human cancers may in fact be due to laboratory contamination by plasmids containing SV40 sequences [18, 19].

2.3. Chromosomal Abnormalities. A number of chromoso- mal and genetic syndromes have been linked to osteosa- rcoma. Osteosarcoma has been reported in patients with Bloom

syndrome, Rothmund-Thompson syndrome, Werner syndrome, Li-Fraumeni syndrome, and hereditary retino- blastoma [15]. Bloom, Rothmund-Thompson, and Werner [20] syndromes are characterised by genetic defects in the RecQ helicase family. DNA-helicases are responsible for separation of double-stranded DNA prior to replication [21, 22]. Mutations in these genes confer a higher risk of multiple malignancies. A recent study of pretherapeutic biopsy specimens has identified amplifications of chromosomes 6p21, 8q24, and 12q14, as well as loss of heterozygosity of 10q21.1, as being among the most common genomic alterations in osteosarcoma. Furthermore, it was concluded that patients carrying these alleles had a poorer prognosis [23]. Numerical chromosomal abnormalities associated with osteosarcoma include loss of chromosomes 9, 10, 13, and 17 as well as gain of chromosome 1 [24].

2.4. Tumour Suppressor Gene Dysfunction. When human cells are exposed to environmental insults, such as those discussed above, somatic DNA may be damaged. Such DNA damage may not necessarily give rise to a malignant cell line, as there are a number of tumour-suppressor mechanisms in place. These mechanisms may either repair the DNA damage or induce apoptosis of these cells. The p53 and retinoblas- toma (Rb) genes are well-known tumour-suppressor genes. However, tumour suppressor genes may themselves become mutated, resulting in the loss of their protective function. As a result, additional somatic mutations may accumulate, giving rise to a cell line that replicates without restraint. Mutations in both the p53 and Rb genes have been proven to be involved in osteosarcoma pathogenesis [6]. The p53 gene is mutated in 50% of all cancers and 22% of osteosarcomas[24].DNAdamageresultsinphosphorylation of p53, which is constitutively inhibited by Mdm2. Phospho- rylation allows p53 dissociation from Mdm2. p53 exerts its tumour-suppressor effects via the activation of proapoptotic Bax and p21. The latter binds and inactivates G1/S-Cdk and S-Cdk complexes, causing arrest of the cell cycle in G1 [25]. Recently, p53 mutations have been shown to result in impaired DNA repair mechanisms and disrupted antian- giogenesis activity [26]. For osteosarcoma, the prototypi- cal condition of p53 mutation is Li-Fraumeni syndrome. This syndrome is characterised by an autosomal dominant mutation of p53 leading to the development of multiple cancersincludingosteosarcoma[27].Li-Fraumenisyndrome and germ-line mutations of p53 in osteosarcomas are rare, however [28], and in many osteosarcoma cell lines, a mutation in the first intron of the p53 gene occurs [29] though other point mutations have also been reported [30]. While p53 has been implicated in the oncogenesis of osteosarcoma, it is unclear whether p53 mutation or loss may affect tumour behaviour. Using the p53-null SaOS-2 osteosarcoma cell line, Ganjavi et al. [31] showed that adenoviral-mediated gene transfer of wild-type p53 resulted in reduced cell viability and increased sensitivity to chemotherapeutic agents. A recent study published by Hu et al. [32] showed that p53 expression was higher in low Rosen grade osteosarcomas (Rosen grade 1: <50% necrosis; grade 2: 50%–90% necrosis; grade 3: >90% necrosis; grade 4: 100% necrosis; grade 1 + 2 = low-grade; grade 3 + 4 = high grade). p53 expression correlated with reduced metastatic disease and improved survival for these patients. p53 mutation has also been shown to be more common in high-grade conven- tional osteosarcomas versus low grade central osteosarcomas [33]. However, other studies differ such as that of Lonardo et al. [34], which found no relationship between p53 and histological grade. Univariate analysis performed by Park et al. [35] showed no correlation between survival and the p53 protein, while coexpression of p53 and P-glycoprotein was associated with a poorer prognosis. In addition to p53, the Rb tumour suppressor has also been implicated in the tumorigenesis of osteosarcoma. The Rb gene is critical to cell-cycle control, and inherited mutation of the Rb gene causes retinoblastoma syndrome, a conditionthatpredisposesapatienttomultiplemalignancies including osteosarcoma. The Rb

protein regulates the cell cycle by binding the transcription factor E2F. E2F is held inactive by Rb until the CDK4/cyclin D complex phospho- rylates Rb. Mutations of Rb allow for the continuous cycling of cells [25]. Both germ-line and somatic mutations of Rb confer an increased risk of osteosarcoma. Loss of the Rb gene may even explain the familial risk of osteosarcoma [36]. However, it hasyettobe determinedwhetherRbgenelossorsuppression gives rise to more aggressive tumours with poorer prognosis. Loss of heterozygosity for Rb has been reported to confer both an improved and poorer prognosis for patients [37–40]. In terms of response to chemotherapeutic treatment, Iida et al. [41] showed that the SaOS-2 osteosarcoma cell line, lack- ing active Rb, was less sensitive to the growth-suppressing effect of methotrexate compared to cell lines with wild-type Rb gene. Further studies are warranted to investigate the role of Rb on chemosensitivity of osteosarcoma cells.

2.5. Transcription Factors. Transcription is the process of forming single-stranded messenger RNA (mRNA) sequences from double-stranded DNA. Transcription factors facilitate binding of promoter sequences for specific genes to initiate the process. While transcription is usually tightly regulated, deregulation may occur in osteosarcoma, as with other cancers. Excess production of transcription factors, or the production of a new overactive transcription factor, may result from gene rearrangement. The activator protein 1 complex (AP-1) is a regulator of transcription that controls cell proliferation, differentiation, and bone metabolism. AP-1 is comprised of Fos and Jun proteins, products of the c-fos and c-jun proto-oncogenes, respectively. Fos and Jun are found to be significantly upreg- ulated in high-grade osteosarcomas compared with benign osteoblastic lesions and low-grade osteosarcomas [42, 43] and are associated with the propensity to develop metastases [44]. Fos and Jun double-transgenic mice are found to develop osteosarcomas with a higher frequency than c- Fos only transgenic mice [45]. Most recently, Leaner et al. [46] showed that inhibition of AP-1-mediated transcription caused reduced migration, invasion, and metastasis in a murine model of osteosarcoma. Another approach has been totargettheJuncomponentofAP-1.TheDNAenzymeDz13 cleaves human c-Jun mRNA and is capable of inhibiting osteosarcoma growth and progression in a clinically relevant murine model when delivered by nanoparticle vector [47]. Myc is a transcription factor that acts in the nucleus to stimulate cell growth and division. Myc amplification has been implicated in osteosarcoma pathogenesis and resistance to chemotherapeutics. Overexpression of Myc in bone marrow stromal cells leads to osteosarcoma development and loss of adipogenesis [48]. Myc is amplified in U2OS osteosarcoma cell-line variants with the highest resistance to doxorubicin, and gain of Myc was found in SaOS-2 methotrexate-resistant variants [49]. Additionally, Myc has been examined as a therapeutic target for osteosarcoma. Downregulation of Myc enhanced the therapeutic activity of methotrexate against osteosarcoma cells [50]. Adenovirus- mediated transfection with the antisense Myc fragment led to cell-cycle arrest and enhanced apoptosis in the MG-63

osteosarcoma cell line [51]. Using a conditional transgenic mouse model, Arvanitis et al. [52] showed that Myc inac- tivation caused proliferative arrest and promoted differen- tiation in osteosarcoma. Additionally, using positron emis- sion tomography (PET), these tumours exhibited reduced metabolic activity as demonstrated by reduced uptake of [18F]-fluorodeoxyglucose ([18F]-FDG).

2.6. Growth Factors. Osteosarcoma cells produce a range of growth factors that exert autocrine and paracrine effects. Dysregulatedexpressionofgrowthfactorssuchastransform- ing growth factor (TGF), insulin-like growth factor (IGF), and connective tissue growth factor (CTGF) leads to the accelerated proliferation of cells. Growth factor receptors may be overexpressed and constitutively activated. Signal transduction associated with these

receptors may also be overactivated. Transforming growth factor beta (TGF-β) proteins are a large family of dimeric proteins secreted by cells. Like many other growth factors, they influence a wide variety of cell process such as di fferentiation, proliferation, apop- tosis, and matrix production. Bone morphogenic proteins (BMPs) make up a large component of the TGF-β family. High-grade osteosarcomas are found to express TGF-β1 in significantly higher amounts than low-grade osteosarcomas [53]. Navid et al. [54] examined the autocrine role of TGF- β on two osteosarcoma cell lines, demonstrating a 30%– 50% reduction in growth when osteosarcoma cells were cultured in the presence of TGF-β-blocking antibody. Smad activation was implicated downstream of TGF-β with an inability to phosphorylate the Rb protein. Most recently, Hu et al. [55, 56] have shown an association between increased susceptibility and metastasis of osteosarcoma with TGFR1 variants, TGFBR1∗6A, and Int7G24A. IGF (insulin-like growth factor)-I and IGF-II are growth factors that are often overexpressed by osteosarcomas. These ligands bind corresponding receptors such as IGF-1R, leading to activation of the PI3K and MAPK transduction pathways. This, then, supports cell proliferation and inhi- bition of apoptosis [57]. The growth-stimulating effect of IGFhasbeentargetedforosteosarcoma.Lentivirus-mediated shRNA targeting IGF-R1 enhanced the chemosensitivity of osteosarcoma cells to docetaxel and cisplatin [58]. The use of monoclonal antibodies targeting IGF-R1 was also effective in enhancing antitumour response [59, 60]. Connective tissue growth factor (CTGF) is related to a number of proteins in the CCN family (CTGF/Cyr61/ Cef10/NOVH). This protein family appears to act via inte- grin signalling pathways [61] and, like TGF-β, has a diverse range of functions including adhesion, migration, prolifer- ation, survival, angiogenesis, and differentiation. Nishida et al. [62] showed that CTGF is a potent stimulator for the proliferation of SaOS-2 cells, leading to increased expres- sion of type I collagen, alkaline phosphatase, osteopontin, and osteocalcin, markers for bone cell differentiation and maturation. A related protein, CCN3, was found to be overexpressed in osteosarcoma and associated with a worse prognosis [63].

Parathyroid hormone (PTH), parathyroid hormone- related peptide (PTHrP), and the receptor (PTHR1) have been implicated in the progression and metastasis of osteosarcoma. PTHrP was discovered as the humoral factor associated with tumour metastasis and hypercalcaemia [64]. The role of PTHrP and PTHR1 in osteoclast signalling will be discussed later. In terms of direct effects on osteosarcoma cells, when HOS osteosarcoma cells were overexpressed with PTHR1, increased proliferation, motility, and invasion through Matrigel were observed [65]. Gagiannis et al. [66] recently showed that PTHrP confers chemoresistance in osteosarcoma by blocking signalling via p53, death- receptor and mitochondrial pathways of apoptosis. PTHrP downregulated expression of proapoptotic Bax and PUMA and upregulated antiapoptotic Bcl-2 and Bcl-xl. Berdiaki et al. [67], using MG-63 and SaOS-2 osteosarcoma cell lines, showed that PTH peptides enhanced osteosarcoma cell migration through the regulation of hyaluron metabolism. However, a previous study showed that overexpression of PTHrP in a murine osteoblastic osteosarcoma cell line re- duced cell proliferation by 80% [68]. Further studies are required to determine the prognostic significance of PTH/ PTHrP/PTHR1 signalling in osteosarcoma.

2.7. Osteosarcoma Cell Proliferation, Apoptosis, and Anchora- ge-Independent Growth. Cancer cells are relatively resistant to apoptosis, and this ability to avoid elimination contributes to the ability of osteosarcoma cells to proliferate without restriction. Apoptosis consists of initiation and execution phases. During initiation, enzymes responsible for the

cleavage of vital cellular proteins, known as caspases, are activated. Execution refers to the actual process of hydrolysis performed by activated caspases. Both extrinsic and intrinsic pathways regulate the initiation phase. The extrinsic pathway is a death receptor-initiated

pathway, while the intrinsic pathway relies on increased mitochondrial permeability. Both proapoptotic and antiapoptotic factors interact with these pathways, and these have been discussed in a previous review [69]. Anoikis is a form of apoptosis that is induced when cells are no longer attached to a basement membrane or matrix. This is of particular interest in osteosarcoma given the propensity of osteosarcoma cells to detach from matrix components and to metastasise. Osteosarcoma cells are resistant to anoikis and proliferate despite deranged cell-cell and cell-matrix attachments. This resistance to anoikis is termed anchorage-independent growth (AIG). The pathways causing anoikis disruption and leading to anchorage-independent growth are complex. They involve interactions between integrin signalling, Rho GTPases, PI3 kinase, and PKB/Akt activation, along with many key componentsoftheintrinsic andextrinsicapoptosis pathways (Figure 1). For example, when normal cells adhere to sur- rounding matrix via integrin-fibronectin binding, the Bcl-2 inhibitor Bit1 is suppressed allowing Bcl-2 to prevent apop- tosisviatheintrinsicpathway[70].Anotherpathwayinvolves the exchange of integrin subunits resulting in the production of abnormal integrins, such as αvβ6, which can upregulate PI3 kinase function [71]. PI3 kinase can then activate PKB/Akt which inhibits the proapoptotic factor Bad, leading to cancer cell survival [72]. Rho GTPases such as Rac1 and Cdc42 can also upregulate PI3 kinase with similar conse- quences [73]. Increased epidermal growth factor-receptor (EGF/EGFR) binding with subsequent extracellular signal- regulated kinase (Erk)/microtubule-associated protein kina- se (MAPK) signalling leading to inhibition of Bim has also beendescribed[74]. This suppressescelldeath, asBim would normally act to increase mitochondrial outer membrane permeability allowing release of cytochrome c and then the activation of executioner caspases.

2.8. Tumour Angiogenesis. Tumour angiogenesis is essential for sustained osteosarcoma growth and metastasis. Without a supporting vasculature, osteosarcoma cells would be unable to obtain the nutrients and oxygen necessary for proliferation. Metastasis to the lungs and bone, the most common sites for osteosarcoma spread, also relies on the formation and maintenance of blood vessels. Radiation therapies, while compromising tumour cells, also destroy the vascular component of tumours and block the supply of nutrients. So, radio- and chemotherapies act by these dual actions. This aspect is discussed below. A balance between pro-angiogenic and antiangiogenic factors regulates angiogenesis, and this balance is tipped towards the favour of neovascularisation by tissue hypoxia, acidosis, oncogene activation, and loss of tumour suppressor gene function. A hypoxic and acidotic microenvironment exists around proliferating osteosarcoma cells, and these conditions stimulate deubiquitination of von Hippel Lin- dau protein. Von Hippel Lindau protein releases hypoxia- inducible factor-1α (HIF-1α), allows HIF-1α to bind to the promoter region of the vascular endothelial growth factor (VEGF) gene [75], and upregulats it. TGF-α, and fibroblast growth factor (FGF) may also upregulate VEGF [76]. VEGF is the best- characterised pro-angiogenic factor, and it stimulates the processes of endothelial cell prolifera- tion, migration, and blood vessel maturation. A number of different VEGF molecules exist (VEGF-A through to VEGF- E), and these proteins bind to VEGF receptors (VEGFR1- 3) [77]. VEGF-A has the broadest angiogenic effect. Upon VEGF-A binding to VEGFR2, a number of divergent sig- nalling pathways are initiated [77]. Nitric oxide (NO) is released by endothelial cells, leading to vasodilation and increased vascular permeability [78]. Endothelial cell pro- liferation and cycling are stimulated via phospholipase Cγ (PLCγ), protein kinase C (PKC), and the c-Raf-MEK-MAPK cascades [77]. Rearrangement of the actin cytoskeleton, necessary for endothelial cell migration occurs via phospho- rylation of T cell-specific adapter (TSAd) and interaction with Src, another protein kinase [79]. The net result of all these changes is the formation of an immature, irregular, and leaky vascular

network. Theimmatureandinefficientnatureofthevesselssopro- duced facilitates feedback loops for further vessel formation. Upregulation of HIF-1α and VEGF [80] again occurs as the leaky vasculature is unable to meet the metabolic demands of the proliferating osteosarcoma cells. Additionally, VEGF upregulates matrix metalloproteinase (MMP) and plasmin activity [81]. These proteases break down extracellular

matrix, which releases any VEGF combined with heparin proteoglycan in the matrix. VEGF also induces antiapoptotic factors Bcl-2, and survivin, ensuring ongoing endothelial proliferation [82]. In addition to VEGF, the proliferating tumour cells release a number of other pro-angiogenic factors. These include FGF, platelet-derived growth factor (PDGF), angiopoietin1 (Ang1), and ephrin-B2 [83, 84]. While it is known that osteosarcoma is a relatively vascular tumour, the prognostic significance of this is yet to be determined. There have been studies suggesting both a correlation [85, 86] and lack of association [87] between VEGF expression and osteosarcoma microvascular density and metastases at diagnosis. This may relate to a greater tumour dependence on functionally mature vessels. One study that demonstrated a survival advantage associated with increased osteosarcoma microvascular density [88] attributed this advantage to improved tissue penetration by chemotherapeutic agents. Aspreviouslymentioned,angiogenesisisregulatedbythe balance between pro-angiogenic and antiangiogenic factors. Antiangiogenic proteins such as thrombospondin 1, TGF-β [89], troponin I, pigment epithelial-derived factor (PEDF) [90], and reversion-inducing cysteine rich protein with Kazal motifs (RECK) [91] are downregulated in osteosarcoma. These antiangiogenic molecules are particularly important for embryogenesis and physiological processes such as wound healing and menstruation; however, they also play a protective mechanism against osteosarcoma progression. For example, troponin I and PEDF are expressed predominately within the avascular zones of the cartilaginous growth plate [92, 93] and are likely to contribute to growth plate resistance to osteosarcoma invasion from a typical metaphyseal location. In addition to inhibiting angiogenesis, PEDF exerts direct effects on osteosarcoma cells. Ek et al. [94, 95] have demonstrated apoptosis induction in osteosarcoma cell lines treated with PEDF. Also, in a murine model of orthotopic osteosarcoma, tumour volume was reduced by PEDF, which was associated with reduced microvascular density. There was decreased tumour metastases and reduced size of metastatic tumours in lung.

2.9. Cell Adhesion and Migration. Osteosarcoma is a highly metastatic tumour, and pulmonary metatases are the most common cause of death. The metastatic sequence involves the detachment of osteosarcoma cells from the primary tumour, adhesion to the extracellular matrix, local migration and invasion through stromal tissue, intravasation, and extravasation.Theabilityofosteosarcomacellstometastasise by such a pathway relies on complex cell-cell and cell-matrix interactions. The extracellular matrix is composed of various protein fibrils and growth factors. The proteins include fibronectin, collagens, proteoglycans, and laminins. Osteosarcoma cells may also produce matrix proteins. The extracellular matrix provides a developing tumour with a supporting scaffold and facilitates blood vessel formation. Osteosarcoma cells adhere to matrix components via cell-surface receptors. These receptors are more than just a physical point of attachment; they also provide a link between matrix proteins and the cytoskeleton. The principle receptor proteins are the integrins, which bind to the matrix protein fibronectin. There are 24 different integrin heterodimer molecules con- sisting of different α and β subunits [96]. Theintegrinsalsoplayaroleincellsignaling,particularly in pathways critical to cell migration. Integrin-binding proteinssuchastalinbecomeassociatedwiththecytoplasmic domain and act, via adaptor proteins such as vinculin, paxillin, and α-actin, for the upregulation of protein kinases [97]. The key enzymes involved here are focal adhesion kinase (FAK), protein kinase C

(PKC), PI3 kinase, Src, and the RhoA GTPases. The relative activities of these enzymes underlie con- formational changes in cell architecture. For example, there is a shifting balance between two of the RhoA GTPases: Rac1 and RhoA. High Rac1 expression suppresses RhoA and induces the formation of membrane ruffles. These mem- brane changes facilitate cell spreading and migration [98]. Conversely, high RhoA with low Rac1 leads to membrane retraction. These two processes are coordinated such that in cell migration, the leading edge of the cell is demonstrating actin polymerisation and lamellipoedia, while the trailing edge is undergoing actin disassembly. Inhibition of RhoA pathways has been shown to reduce osteosarcoma cell migration and invasion [99]. In general, cells migrate towards ligand-dense matrix and towards more rigid matrix [100], indicating a constant intracellular response to extracellular adhesion and tension. Tumour stroma is more rigid than normal connective tissue matrix, and this generates integrin clustering, activation of intracellular signalling pathways, decreases cell-to-cell contacts, and stimulates tumour growth [101]. The ezrin protein also has a role in cell-cell interactions, signal transduction, linkage between actin filaments, and cell membrane receptors such as CD44, which binds hyaluronan in the extracellular matrix. When ezrin is overexpressed, it is associated with an increase in metastasis [102]. Increased ezrin expression in paediatric osteosarcoma patients is asso- ciated with reduced disease-free intervals, and downregu- lation of ezrin expression in a mouse model of human osteosarcoma has been shown to reduce pulmonary metas- tasis [103].

2.10. Tumor Invasion. Invasion of the surrounding tissues by osteosarcoma also involves degradation of the extracellular matrix. Matrix metalloproteinases (MMPs) are principally involved in the breakdown of the extracellular matrix, alt- hough roles in tumour angiogenesis have also been estab- lished. MMPs are a family of zinc-dependent endopeptidases that are involved in a range of physiological processes includinginflammation,woundhealing,embryogenesis,and fracture healing. In normal tissues, MMPs are regulated by natural inhibitors such as tissue inhibitors of MMPs (TIMPs), RECK, and α2 macroglobulin [104]. In the setting of osteosarcoma, MMPs break down extracellular collagens, facilitating both tumour and endothelial cell invasion. MMPs may be designated as gelatinases, collagenasesm, or stromeolysins. Gelatinases break down denatured collagens

and type IV collagen. Collagenases break down type I, type II, and type III collagen, and stromeolysins break down proteoglycan (found in articular cartilage), type III, type IV (in basement membranes), and type V collagen, as well as casein and fibronectin [105]. In addition to clearing a pathway for invading osteosar- coma cells, the role of MMPs in angiogenesis has already been mentioned. Remodelling of vessel walls by MMPs gives rise to a thin and leaky vascular network that allows passage of tumour cells into the bloodstream [106]. Furthermore, MMP-9 releases VEGF stored within the extracellular matrix [107], and VEGF is able to upregulate MMP-2 [108]. The specific importance of the gelatinases MMP-2 and MMP-9 to tumour progression has been delineated in an in vivo study, where combined MMP-2/MMP-9 deficiency in mice signifi- cantly impaired tumour angiogenesis and invasion [109]. The urokinase plasminogen activator (uPA) system is the otherkeyregulatorofosteosarcomainvasion,whichinteracts with MMPs. The ligand uPA binds to its receptor uPAR to become active. Once activated, uPA cleaves plasminogen to plasmin. Plasmin breaks down the extracellular matrix but also activates pro-MMPs. A cascade of activation is hence established [110, 111]. The role of the uPA-uPAR system is well established in osteosarcoma pathogenesis. An inverse relationship between uPA levels and survival time has been demonstrated [112]. Downregulation of uPAR in anin vivo osteosarcoma model resulted in reduced primary tumour growth and fewer metastases [113].

2.11. Osteoclast Function. Osteosarcoma invasion of bone reliesoninteractionsbetweenthebonematrix,osteosarcoma cells, osteoblasts, and osteoclasts (Figure 2). Osteoclasts are the principle bone-resorbing cells, and the substantial osteolysis exhibited by some osteosarcomas is the direct result of increased osteoclastic activity. During the initial stages of osteosarcoma invasion, growth factors such as TGF-β are released from the degraded bone matrix and act on osteosarcoma cells, stimulating the release of PTHrP, interleukin-6 (IL-6) and interleukin-11 (IL-11) [114, 115]. These cytokines then stimulate osteoclasts, facilitating fur- ther invasion and release of proresorptive cytokines. Osteoblasts are, in fact, mediators in this process of bone resorption. Osteosarcoma cells release endothelin-1 (ET-1), VEGF, and PDGF in response to the hypoxic and acidotic conditions. These factors have predominantly osteoblast- stimulatory functions [116, 117]. PTHrP and IL-11 also act on osteoblasts, stimulating increased expression of receptor activator of nuclear factor κB ligand (RANKL). RANKL is a key mediator of osteoclast differentiation and activity, and osteosarcoma cells have been noted to produce RANKL independently [118]. RANKL activates osteoclasts through binding to RANK on the osteoclast surface. RANK expression is under control of cytokines IL-1, IL-6, IL-8, tumour necrosis factor-α (TNF-α), PTHrP, and TGF-α [119]. Receptor-ligand binding initiates a cascade of events through binding of TRAF-6, leading to activation of both NFκB and MAPK pathways, with a resulting increase in nuclear factor of activated T- cells (NFATc1) activity. RANK/RANKL also activates the

c-Fos component of AP-1, resulting in additional NFATc1 upregulation. NFATc1 is thus a common end-point for effecting transcription of genes involved in osteoclast activity and maturation [120]. Activated osteoclasts release proteases to resorb the nonmineralised components of bone. Cathepsin K (Cat K) is a cysteine protease selectively produced by osteoclasts for breakdown of collagen I, osteopontin, and osteonectin [121]. Cat K is also produced by some cancer cells to aid invasion [122]. This protease is essential for osteoclast function in normal bone remodelling and also in pathological states of osteolysis. For patients with high-grade metastatic osteosar- coma, low Cat K levels at the time of diagnosis confers a better prognosis [123]. c-Src is a nonreceptor tyrosine kinase present within osteoclasts [124] and is involved in pathways regulating cell growth, survival, and migration [125]. Osteoclast survival occurs through c-Src mediating phosphatidylinositol 3- kinase and TRAF-6 interaction, with resulting Akt/mTOR (mammalian target of rapamycin) pathway activation and then inhibition of caspase-3 [126]. Podosome assembly, vesicle transport, secretion of proteases, and organisation of microtubules are all regulated by c-Src pathway activity [127]. For osteosarcoma, inhibition of c-Src induces apopto- sis and inhibits invasion in vitro. Primary tumour volume in a murine model of osteosarcoma was also reduced by c-Src inhibition [128]. Osteoclast pathways of differentiation, maturation, and activation have potential as therapeutic targets. Inhibition of bone resorption at the tumour-bone interface may lead to reduced local invasion by osteosarcoma. The central role that RANKL plays in osteoclast function makes it a particularly attractive target. Osteoprotegerin (OPG) is a soluble decoy receptor for RANKL and strongly suppresses osteoclast differentiation both in vitro and in vivo [129].

OPG gene therapy has been applied to a murine model of osteosarcoma and successfully suppressed osteolytic activity. There were a reduced number of osteoclasts associated with tumours, leading to reduced local osteosarcoma progression and improved survival [130].

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